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INFLUENCE OF CARBONATES OF MAGNESIUM AND CALCIUM ON BACTERIA OF CERTAIN WISCONSIN SOILS[1]

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THE PROBLEM

In the course of a study of the biology of certain acid soils it was found that magnesium carbonate causes a great increase in the reproduction of soil bacteria. Just what effect this great increase in number of bacteria has on the fertility of the soil is a problem beyond the scope of this paper. However, it is a well-established fact that the majority of the changes of soil constituents are brought about by microorganisms. These substances, which are constantly undergoing changes, are influenced by the number and kinds of bacteria.

The beneficial effect of lime, calcium carbonate, and magnesium carbonate on the growth of higher plants is generally preceded by an increase in the number of bacteria. Evidence is sufficient to warrant the conclusion that an increase in the number of soil microorganisms is usually reflected in a more rapid decomposition of organic matter and a greater liberation of the inorganic soil constituents, chiefly potassium and phosphorous.

Although many important data have been obtained in relation to the effect of liming on the bacteria in soil, there still remain problems which deserve careful investigation—for example, the amount and kind of lime and calcium and magnesium carbonates that can be applied most economically and yet give the best results.

The investigations of this paper were undertaken with the hope of throwing some light upon the problem of soil bacteria and their response to applications of magnesium carbonate, calcium carbonate, and limestone.

The effect of these substances on pure cultures of bacteria and on the ordinary soil flora was observed in—

(*a*) Acid Colby silt loam soil;
(*b*) Acid Plainfield sand;
(*c*) Neutral Miami silt loam.

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[2] The writer is indebted to Dr. E. B. Fred, of the Wisconsin Experiment Station, for supervision and suggestions in the experimental work and preparation of the manuscript.

REVIEW OF LITERATURE

A complete review of the literature, showing the effect of lime (CaO), calcium carbonate ($CaCO_3$), and magnesium carbonate ($MgCO_3$) on soil microorganisms, is not attempted in this paper. Only a brief résumé of certain of the more important papers is given.

Although this paper is primarily concerned with the relation of soil microorganisms to calcium and magnesium carbonates, it was deemed worth while to include a brief review of results obtained from the use of lime and limestone. Under ordinary soil conditions, calcium oxid, or lime, is soon converted into calcium carbonate. Because of the frequent occurrence of magnesium in limestone, this substance was included in the discussion.

NUMBER OF ORGANISMS

In 1901 Chester (*7*)[1] made plate counts of an acid soil which had received lime at the rate of 1,000 to 4,000 pounds per acre. In every case the lime increased the total number of bacteria; the greatest gain was noted where the largest amount of lime was applied.

Fischer (*18*) treated soil with lime and calcium carbonate at the rate of 0.1 to 3.0 per cent by weight of soil. The calcium carbonate and lime were added in gram-molecular equivalents. His results showed that 0.1 and 0.2 per cent of lime after three days gave an increase in the total number of bacteria. Treatments amounting to 0.5 per cent and more were harmful at first, but later gave an increase beyond that of the control. The data showed that a slight increase in the number of bacteria occurred when calcium carbonate was added to the soil.

Several years later, Hutchinson (*23*) made somewhat similar experiments, using lime and calcium carbonate. He also found that lime at first exerted a depressing effect but later gave rise to an increase in the number of bacteria. He concluded that the reduction in the number of bacteria immediately after treatment with lime was due to the caustic effect of the oxid, since no injury was noted after the oxid changed to carbonate. He believed the benefit from liming was due in part to the gain in soluble organic matter, to the improvement in the physical condition, and to the correction of the acid reaction of a soil, all of which tended to bring about a better environment for the development of bacteria. However, this investigator held that these changes brought about by the action of liming did not seem sufficient to account for the enormous increase in plant growth. He assumed, therefore, that the action of lime was much the same as an antiseptic—that is, it caused a partial sterilization.

In a later publication, Hutchinson and MacLennan (*24*) reported the results of experiments with lime and calcium carbonate on five different soils. The range of reaction in these soils varied from neutral to strongly

[1] Reference is made by number (italic) to "Literature cited," pp. 500-504.

acid. Lime was added in amounts varying from 0.1 to 1.0 per cent, while calcium carbonate was added in amounts of 1.0 per cent only. Besides the total number of bacteria, these investigators determined the nitrate and ammonia nitrogen of the soils subjected to this treatment. The results of the experiments showed that in all of the soils, liming increased the number of bacteria. At first the heavier applications of lime retarded and later stimulated the reproduction of microorganisms. The acid soil required more lime to retard the growth of bacteria than the nonacid soils. As a result of liming, ammonification and nitrification in all of the soils was increased. The authors concluded that different soils varied in relation to the amount of lime needed to effect partial sterilization. They assumed that each soil absorbed a certain amount before the antiseptic action began.

Miller (*51*) carried out a rather intensive study of the effect of lime and calcium carbonate in both calcium-rich and calcium-poor soils, and also in soils low and high in organic matter. His results were in accordance with those obtained by many other investigators—namely, that lime in small amounts increased the number of bacteria, while larger application, decreased at first and later increased the number. To this investigator it appeared that lime was a direct stimulant to the soil flora. The same general increase was obtained with soil low and soil high in calcium content, and also in soils low and high in organic matter. Unlike lime, calcium carbonate brought about only a slight increase in the number of bacteria.

Beckwith, Vass, and Robinson (*3*) applied lime at the rate of 2 tons per acre to both acid and nonacid soils. Only the soils with an acid reaction or where large amounts of organic matter were added showed an increase in the number of bacteria from the lime treatment. Under the same conditions ammonification and nitrification responded in a like manner.

Soil was treated with lime, magnesium oxid, and magnesium carbonate by Lemmermann and Fischer (*34*). They found that magnesium oxid caused a greater increase in the number of bacteria than did either lime or magnesium carbonate.

Engberding (*13*) treated clay soil with 0.1 per cent of lime and with 0.5 per cent of magnesium oxid. Both treatments increased the number of bacteria, although the lime seemed to give better results.

Jenkins and Britton (*26*) showed that by using heavy applications of lime with raw-bone meal, the latter was decomposed more rapidly.

Fabricius and Van Feilitzen (*14*) noted an increase in the number of bacteria in moor soils treated with lime.

Lemmermann, Fischer, Kappen, and Blanck (*35*) reported a gain in the number of bacteria in cultivated and noncultivated moor soils and in clay soils when lime or calcium carbonate was added. However, an increase in the number of bacteria was not shown in a sandy clay soil or in a sandy soil.

AMMONIA AND NITRATES

Withers and Fraps (*62*, *63*) added calcium carbonate to soil treated with nitrogenous material and observed a gain in nitrates which was greater in the calcium-carbonate soil than in the control. Koch (*31*) reported similar results by applying lime. Fraps (*19*) treated acid and nonacid soils with calcium and magnesium carbonates and noted an increase in nitrates in all cases, especially in the acid soil. However, calcium carbonate gave better results than were obtained with magnesium carbonate.

By applying calcium carbonate to a soil treated with ammonium sulphate, Lemmermann, Blanck, Heinitz, and Von Wlodeck (*36*) noticed a loss in ammonia. Lipman, Brown, and their associates (*4*, *5*, *42*, *43*) studied the effect of calcium carbonate on the actitivies of soil microorganisms. Lipman and Brown (*44*) showed an increase in ammonification with monocalcium phosphate [$CaH_4(PO_4)_2$] and dicalcium phosphate [$Ca_2H_2(PO_4)_2$], but a decrease with tricalcium phosphate [$Ca_3(PO_4)_2$] when applied to soil.

Wohltmann, Fischer, and Schneider (*64*) inoculated liquid media with soil treated with magnesium oxid and lime and observed an increase in both ammonification and nitrification. Hutchinson (*22*) observed that in liquid cultures magnesium carbonate retarded nitrification in practically every case, while calcium carbonate had but little influence on this process.

Vogel (*57*) inoculated a nitrogenous solution containing calcium carbonate with a soil suspension and obtained an increase in ammonia. A similar test for nitrification was made, but an increase in nitrates was not obtained. Somewhat similar results were obtained by Paterson and Scott (*53*). Hutchinson and McLennan (*25*) reported that calcium carbonate treatments caused a slight increase in nitrification in soil.

Greaves (*21*), working with a Utah soil high in lime and magnesium oxid, found that calcium carbonate in all concentrations increased the formation of ammonia, while magnesium carbonate retarded ammonification except when applied in very small amounts. The chlorids of magnesium and calcium appeared to be very toxic. With a Japanese soil Machida (*48*), of the Japanese Experiment Station at Tokio, noted that calcium chlorid retarded ammonification, while magnesium chlorid increased it. He found that nitrification was favored more by the use of magnesium carbonate than by calcium carbonate.

The work of McBeth and Wright (*49*) showed that the chlorids, the sulphates, and especially the carbonates inhibited nitrification. Lyon and Bizzell (*47*) reported that 10 days after treatment with lime the number of bacteria was doubled.

Fred and Graul (*20*) concluded that the accumulation of nitrates from casein or gelatin in acid soils was not materially benefited by calcium carbonate. In many cases in acid soil calcium carbonate increased and

later decreased the nitrate content, and it was assumed that a loss of nitrate nitrogen occurred because of the increase in the total number of microorganisms.

Allen and Bonazzi (*1*) showed that the addition of ground limestone to a noncalcareous soil brought about a more rapid nitrification. Kelley (*28*) studied the effects of calcium and magnesium carbonates on ammonification and nitrification in a California soil high in basic substances. His results showed that calcium carbonate benefited ammonification slightly and nitrification to a great extent, while magnesium carbonate was toxic to both processes. This investigator failed to show any definite ratio of calcium and magnesium which favored the nitrifying and ammonifying power of this soil. With Hawaiian soils, high in lime and magnesium oxid, Kelley (*29*) obtained similar results. Dolomitic and calcareous limestones gave results similar to those obtained with calcium carbonate.

Kellerman and Robinson (*27*) obtained nitrification of ammonium sulphate in a soil with a high magnesium content. When calcium carbonate was added, an increase in nitrification was noted; with magnesium carbonate a decrease, except when the latter substance was added to the soil in very small amounts. Owen (*52*) reported that magnesium carbonate favored nitrification more than calcium carbonate.

White (*61*) and Voorhees and Lipman (*58*) treated soil with pure lime and with a lime containing magnesium. Better nitrification and in most cases better ammonification was obtained from the magnesium than from the nonmagnesium limed soil. Soil treated with magnesium carbonate and inoculated into a liquid medium was shown by Lipman and Brown (*41*) to retard nitrification.

It was shown by Ehrenberg (*12*), Lemmermann, Aso, Fischer, and Fresenius (*37*), and Wheeler, Sargent, and Hartwell (*60*), that when lime or calcium carbonate was applied to soil, the decomposition of organic matter was accelerated.

NITROGEN FIXATION

Fischer (*15*, *16*) demonstrated the fact that both lime and magnesium oxid increased the reproduction of *Bacillus azotobacter* in soil. The oxid of magnesium seemed to give better results than lime.

So much is *Bacillus azotobacter* influenced by lime that Christensen and Larsen (*9*) suggested the use of this organism to measure the reaction of soil. In a later publication Christensen (*8*) showed that the growth of this organism in solution took place only when inoculated with a basic soil. In their work with Danish soils, Weis and Bornebusch (*59*) confirmed Christensen's results. Loew (*45*) showed that lime added to a soil increased the growth of the film of *B. azotobacter* formed in liquid cultures. Results similar to Loew's were obtained by Cauda (*6*) when calcium carbonate was used.

Fischer (*16*) obtained a better growth of *Bacillus azotobacter* from limed than from unlimed clay soil. On the other hand, Koch, Litzendorf, Krull, and Alves (*32*) reported that lime retarded free-nitrogen fixation. Krüger (*33*) treated soil with lime and obtained an increase in nitrogen fixation.

Purer and better film growth of *Bacillus azotobacter* was obtained by Ashby (*2*) with magnesium carbonate than with calcium carbonate.

The effect of calcium carbonate and magnesium carbonate on the fixation of nitrogen by *Bacillus azotobacter*, both in liquid cultures and in soil, was studied by Lipman and Burgess (*40*). In every case magnesium carbonate alone proved toxic. These authors observed that when 15 parts of calcium carbonate were mixed with 1 part of magnesium carbonate, the latter was no longer toxic to this organism.

SUMMARY OF LITERATURE

From the citations just given it appears that the addition of calcium and magnesium, either in the form of oxid or carbonate, to soil, and especially to acid soil, brings about conditions favorable to the growth of certain groups of microorganisms. There are many factors which have been given little or no consideration—for instance, what relationship exists between the total number of bacteria in soil and the quantity of soil acid neutralized? With few exceptions, little attention has been directed toward the relative effect of calcium and magnesium carbonates on the soil flora. There exists a diversity of opinion with regard to the relation of bacteria to these two compounds. This lack of harmony may be due to the difference in the soil types which have been studied.

Again, it seems that no one has tried to measure the effect of calcium and magnesium carbonates on pure cultures of bacteria in sterilized acid soil. To obtain information with regard to these points, a series of experiments was planned.

EXPERIMENTAL WORK

For this study three Wisconsin soils, acid Colby silt loam, acid Plainfield sand, and neutral Miami silt loam, were used. The Colby silt loam was collected near Marshfield, the Plainfield sand from Hancock, and the Miami silt loam from Madison. At the laboratory each soil was passed through a 4-mm. sieve and thoroughly mixed.

The percentage composition of these soils is given below:

Constituent.	Colby silt loam.	Miami silt loam.	Plainfield sand.
Potassium	1.51	2.16	0.93
Nitrogen	.198	.15	.09
Phosphorous	.072	.15	.032
Calcium oxid	.0907	.185	.0023
Organic matter	3.91	2.74	1.41

The calcium-carbonate requirement of the acid soils was determined according to the Truog barium-hydroxid method. In calculating the amount of acid in each soil only the active acidity was considered. For every 100 gm. of Colby silt loam on the dry basis 1.05 gm. of calcium carbonate were required to correct the active acidity, and for 100 gm. of Plainfield sand 0.21 gm. Three different bases were used to neutralize the acidity in these soils—namely, pure precipitated calcium carbonate, pure precipitated magnesium carbonate, and commercial ground limestone. The limestone, the analysis of which showed 53 per cent of calcium oxid and 43 per cent of magnesium oxid, was ground to pass through a 100-mesh sieve.

Aside from the compounds just named, monocalcium phosphate was used in certain experiments. The phosphate was employed to find out whether or not the calcium of this phosphate salt would serve in a like capacity as that of calcium carbonate. Accordingly the monocalcium phosphate was added to the soil alone and in various mixtures with calcium carbonate.

The calcium carbonate, magnesium carbonate, and limestone were added in amounts sufficient to satisfy one-fourth, one-half, and full calcium-carbonate requirement—that is, to neutralize one-fourth, one-half, and the total active acidity. The phosphate was added in varying amounts. After the bases and phosphate were thoroughly mixed with the soil, the latter was then poured into earthenware jars and the moisture content raised to one-half saturation with distilled water. At definite intervals samples were drawn and plate counts made. The soil of each jar, after the sample had been drawn, was poured on sterile paper, thoroughly mixed, and returned to the original jar. In order to reduce evaporation and to prevent outside contamination, the jars were covered with cheesecloth. The entire series of jars was incubated in the greenhouse at approximately 22° C.

At regular intervals the effect of these compounds on the total number of bacteria in the soil, on ammonification, and on nitrification was studied.

INFLUENCE OF CALCIUM CARBONATE, LIMESTONE, AND MONOCALCIUM PHOSPHATE ON THE NUMBER OF BACTERIA IN SOIL

COLBY SILT LOAM.—Two-kgm. portions of soil were treated as outlined in Table I and incubated for a period of five months. During this time eight plate counts were made with Heyden-Nährstoff agar. These plates were incubated at 27° C. for 10 days. The influence of monocalcium phosphate on the number of bacteria in the soil was tested simultaneously with that of the carbonates. The data of this experiment are given in Table I.

TABLE I.—*Influence of calcium carbonate, limestone, and monocalcium phosphate on the number of bacteria in Colby silt loam*

Treatment.	Number of bacteria in 1 gm. of dry soil.									
	After 1 week.	Relative.	After 2 weeks.	Relative.	After 3 weeks.	Relative.	After 8 weeks.	Relative.	After 20 weeks.	Relative.
None	18,600,000	100	19,200,000	100	12,800,000	100	13,000,000	100	6,700,000	100
One-fourth calcium carbonate	21,000,000	113	22,400,000	116	21,300,000	166	18,000,000	138	16,400,000	245
One-half calcium carbonate	20,500,000	111	26,000,000	135	14,000,000	109	13,000,000	100	14,700,000	220
Full calcium carbonate	18,100,000	97	21,200,000	110	13,100,000	102	18,000,000	138	13,600,000	202
One-fourth limestone	21,000,000	113	24,500,000	127	15,000,000	117	16,000,000	123	13,500,000	201
One-half limestone	25,300,000	136	19,500,000	101	15,500,000	121	14,900,000	114	11,500,000	173
Full limestone	22,300,000	120	18,100,000	94	20,700,000	161	17,100,000	131	12,700,000	189
0.5 gm. monocalcium phosphate	14,300,000	77	15,500,000	80	18,000,000	140	13,300,000	103	6,400,000	95
2 gm. monocalcium phosphate	22,300,000	120	21,000,000	109	20,200,000	157	13,600,000	104	8,300,000	124
0.5 gm. monocalcium phosphate + one-fourth calcium carbonate	16,000,000	86	15,000,000	78	18,300,000	142	17,200,000	132	17,400,000	260
0.5 gm. monocalcium phosphate + full calcium carbonate	21,200,000	114	31,700,000	165	22,500,000	175	15,400,000	119	14,300,000	213
2 gm. monocalcium phosphate + one-fourth calcium carbonate	14,200,000	76	21,100,000	109	26,200,000	204	15,200,000	117	11,600,000	173
2 gm. monocalcium phosphate + full calcium carbonate	16,100,000	86	20,900,000	108	35,600,000	278	12,500,000	97	11,000,000	164

It will be seen from the figures in Table I that in practically every case calcium carbonate, either pure or in the form of limestone (dolomitic), increased the growth of bacteria to a considerable extent. As compared with the untreated soil, the favorable influence of the calcium compounds on the number of bacteria was greatest 3, 8, and 20 weeks after treatment. Apparently these compounds of calcium, especially the carbonate, have little influence on the soil flora for the first week. This is to be expected, since calcium carbonate is very slowly soluble.

The most striking fact noted from the results of this experiment is the marked stimulation of the microorganisms following small applications of calcium carbonate. Figure 1 is a diagram showing the effect of calcium carbonate and limestone on the total number of bacteria. One-fourth enough calcium carbonate to neutralize the entire soil acidity, with only one exception, showed the greatest increase in the number of bacteria. If grouped according to their effect on the number of soil organisms, one-fourth calcium carbonate gave the greatest gain in the number of bacteria, one-half, the next greatest, and full, the least. The beneficial effect of calcium carbonate extended over the entire period of five months—that is, the treated soil gave a decided increase in the total number of bacteria as compared with the untreated soil. In general, pure calcium carbonate proved superior to limestone in its effect on the bacteria of Colby silt loam soil. This superiority of calcium carbonate

as compared with limestone was due probably to the difference in solubility of the two compounds; pure calcium carbonate is more soluble than the dolomitic limestone.

The monocalcium phosphate in small amounts apparently did not increase the total number of bacteria, whereas in larger amounts, applied alone, it was slightly beneficial. In two instances a combination of calcium carbonate and phosphate showed an increase in the total number of microorganisms. However, in most cases the increase was no greater than that obtained with calcium carbonate alone.

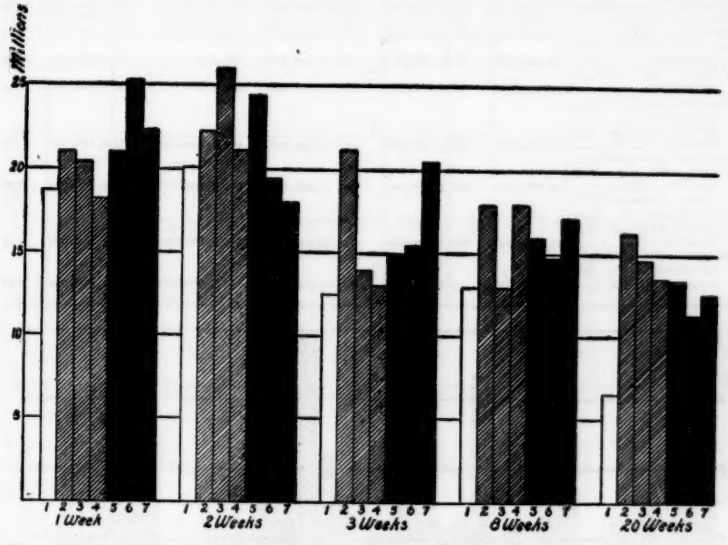

FIG. 1.—Diagram showing the influence of calcium carbonate and limestone on the number of bacteria in Colby silt loam.

1—no treatment.
2—one-fourth cacium carbonate.
3—one-half calcium carbonate.
4—full calcium carbonate.
5—one-fourth limestone.
6—one-half limestone.
7—full limestone.

A comparison of the influence of calcium from calcium phosphate with that from calcium carbonate on the number of bacteria in Colby silt loam is shown in figure 2. Although there are many fluctuations, the results indicate that calcium phosphate alone or with calcium carbonate increases the number of bacteria much sooner than does the carbonate alone. Here the maximum gain with the phosphate was noted 3 weeks after treatment instead of 20 weeks, as in the case of calcium carbonate.

PLAINFIELD SAND.—The preceding experiment was repeated with a Plainfield sand, a soil very low in organic matter. The results obtained in this test are presented in Table II.

TABLE II.—*Influence of calcium carbonate, limestone, and monocalcium phosphate on the number of bacteria in Plainfield sand*

Treatment.	Number of bacteria in 1 gm. of dry soil.									
	After 1 week.	Relative.	After 2 weeks.	Relative.	After 3 weeks.	Relative.	After 8 weeks.	Relative.	After 20 weeks.	Relative.
None	10,300,000	100	3,500,000	100	5,500,000	100	4,700,000	100	2,450,000	100
One-fourth calcium carbonate	10,000,000	97	3,600,000	102	6,000,000	109	6,600,000	140	4,850,000	197
One-half calcium carbonate	6,600,000	64	4,660,000	130	11,200,000	203	7,700,000	164	4 330,000	172
Full calcium carbonate	6,000,000	60	5,520,000	158	18,300,000	332	7,300,000	155	5,800,000	236
One-fourth limestone	5,800,000	56	4,660,000	130	11,000,000	200	8,500,000	181	4,000,000	161
One-half limestone	8,000,000	77	3,800,000	109	10,000,000	180	7,700,000	164	3,000,000	122
Full limestone	9,700,000	94	4,200,000	120	9,700,000	176	7,200,000	154	4,530,000	184
0.5 gm. monocalcium phosphate	8,500,000	84	4,200,000	120	5,700,000	103	4,100,000	87	2,650,000	108
2 gm. monocalcium phosphate	5,000,000	48	4,200,000	120	8,900,000	161	3,200,000	68	2,000,000	82
0.5 gm. monocalcium phosphate + one-fourth calcium carbonate	16,000,000	156	3,100,000	90	14,500,000	263	8,000,000	170	2,120,000	86
0.5 gm. monocalcium phosphate + full calcium carbonate	12,000,000	116	4,200,000	120	9,400,000	170	6,800,000	144	7,770,000	317
2 gm. monocalcium phosphate + one-fourth calcium carbonate	11,200,000	109	2,700,000	80	9,300,000	169	6,600,000	140	3,410,000	139
2 gm. monocalcium phosphate + full calcium carbonate	5,700,000	55	5,200,000	150	10,300,000	187	8,100,000	172	3,660,000	148

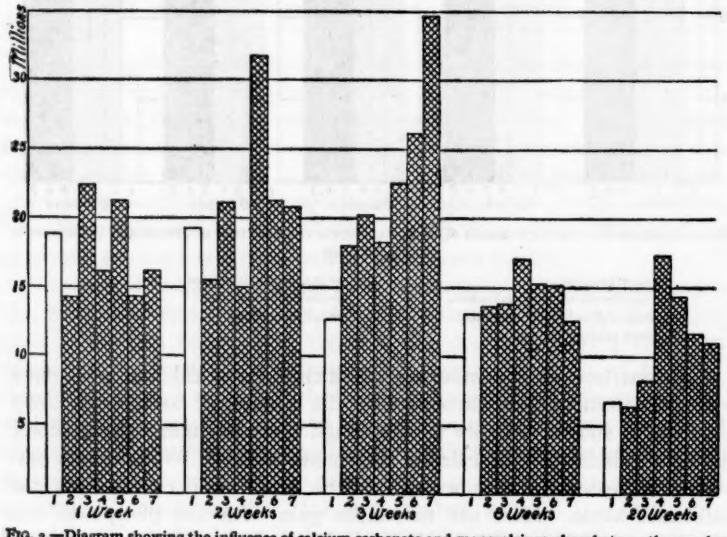

FIG. 2.—Diagram showing the influence of calcium carbonate and monocalcium phosphate on the number of bacteria in Colby silt loam.

1=no treatment.
2=0.5 gm. monocalcium phosphate.
3=2 gm. monocalcium phosphate.
4=0.5 gm. monocalcium phosphate +one-fourth calcium carbonate.
5=0.5 gm. monocalcium phosphate+full calcium carbonate.
6=2 gm. monocalcium phosphate+one-fourth calcium carbonate.
7=2 gm. monocalcium phosphate+full calcium carbonate.

The results reported in Table II differ somewhat from those obtained with Colby silt loam. One week after the treatment there was a decrease in the number of bacteria in the soil treated with both the calcium carbonate and limestone. However, after the first week the treated soil showed an increase in the number of bacteria.

In contrast with the results of the Colby silt-loam experiment, the Plainfield sand, to which one-fourth calcium carbonate or limestone was added, did not give any marked gain in the total number of bacteria after 1 and 2 weeks. After 3, 8, and 20 weeks, one-fourth calcium carbonate caused a slight increase in the number of soil organisms. It is evident that one-half or full neutralization of the soil acids by the calcium carbonate was required to give the greatest increase in the number of bacteria (fig. 3).

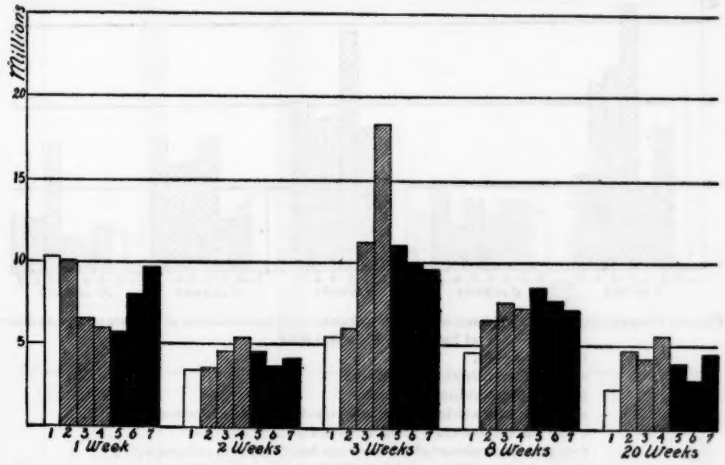

FIG. 3.—Diagram showing the influence of calcium carbonate and limestone on the number of bacteria in Plainfield sand.

1=no treatment.
2=one-fourth calcium carbonate.
3=one-half calcium carbonate.
4=full calcium carbonate.
5=one-fourth limestone.
6=one-half limestone.
7=full limestone.

In general, light applications of limestone gave a greater increase in the number of microorganisms than did calcium carbonate. A difference in the nature of the soil acid in Colby and Plainfield sand probably accounts for the difference in quantity of calcium carbonate required to stimulate the reproduction of bacteria.

Where monocalcium phosphate was added alone to the sandy soil, practically no increase in the number of bacteria was obtained. A combination of the phosphate with calcium carbonate apparently did not stimulate the multiplication of bacteria any better than did calcium carbonate alone except where a combination of 0.5 gm. of monocalcium

phosphate and one-fourth calcium carbonate requirement was used. It is surprising that this combination should favor the development of bacteria more than a heavier application of the same combination (fig. 4).

INFLUENCE OF MAGNESIUM CARBONATE ON THE NUMBER OF BACTERIA IN SOIL

COLBY SILT LOAM.—Since pure calcium carbonate or dolomitic limestone failed to give a large increase in the total number of bacteria, an attempt was made to determine what effect magnesium carbonate would have on the soil flora. Accordingly an experiment was planned in which pure magnesium carbonate was added to the soil. The magnesium carbonate was applied to the soil in the gram-molecular equivalent of calcium carbonate. The procedure in this experiment was similar to that just described. The results of this test are presented in Table III.

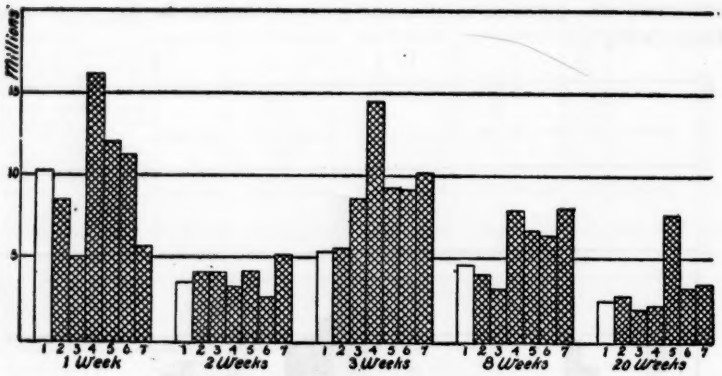

FIG. 4.—Diagram showing the influence of calcium carbonate and monocalcium phosphate on the number of bacteria in Plainfield sand.

1=no treatment.
2=0.5 gm. monocalcium phosphate.
3=2 gm. monocalcium phosphate.
4=0.5 gm. monocalcium phosphate+one-fourth calcium carbonate.
5=0.5 gm. monocalcium phosphate+full calcium carbonate.
6=2 gm. monocalcium phosphate+one-fourth calcium carbonate.
7=2 gm. monocalcium phosphate+full calcium carbonate.

TABLE III.—*Influence of magnesium carbonate on the number of bacteria in Colby silt loam*

Treatment.	Number of bacteria in 1 gm. of dry soil.									
	After 1 week.	Relative.	After 2 weeks.	Relative.	After 3 weeks.	Relative.	After 8 weeks.	Relative.	After 20 weeks.	Relative.
None	25,200,000	100	21,000,000	100	19,300,000	100	14,000,000	100	8,600,000	100
One-fourth magnesium carbonate	36,600,000	145	29,700,000	141	30,500,000	157	17,200,000	122	11,700,000	136
One-half magnesium carbonate	44,800,000	178	41,000,000	195	45,200,000	234	19,400,000	138	12,200,000	141
Full magnesium carbonate	156,000,000	615	125,000,000	595	74,500,000	386	59,100,000	422	26,000,000	302

Unlike calcium carbonate and limestone, full applications of magnesium carbonate increased the number of bacteria far beyond the increase obtained with the one-half and one-fourth treatments. From the figures in this table it will be seen that the effect of the application of magnesium carbonate to Colby soil invariably increased the reproduction of the soil bacteria, especially during the first, second, and third week.

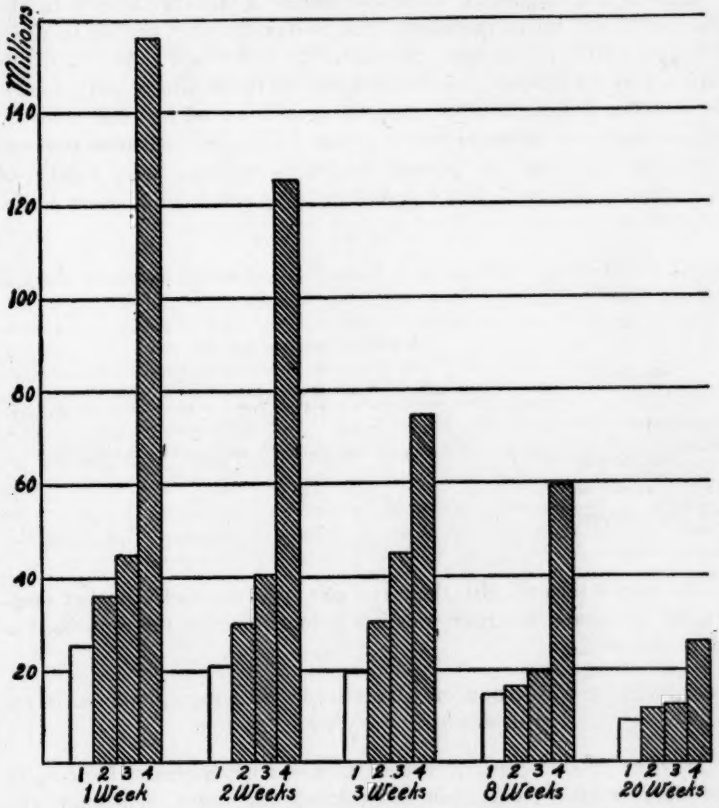

FIG. 5.—Diagram showing the influence of magnesium carbonate on the number of bacteria in Colby silt loam.

1=no treatment.
2=one-fourth magnesium carbonate.
3=one-half magnesium carbonate.
4=full magnesium carbonate.

Figure 5 shows very conclusively the marked effect of magnesium carbonate on the number of soil bacteria. For instance, the increase with full magnesium-carbonate treatment was more than six times as great as that of the control. The absolute numbers varied with the time of the count, but the ratio of numbers between the different quantities of magnesium carbonate remained almost the same throughout the entire

period of 20 weeks. The increase was maintained for a period of 20 weeks, but the total number of organisms decreased greatly during this time.

PLAINFIELD SAND.—It was next arranged to determine the effect of magnesium carbonate on the number of microorganisms in Plainfield sand. The results are summarized in Table IV.

Here again, magnesium carbonate caused a striking increase in the number of soil microorganisms. The results are very similar to those obtained with magnesium carbonate in Colby soil. An enormous increase in the number of soil organisms was noted after 1 and 3 weeks. The sudden drop in numbers after the 3-week period was due probably to a mistake in weighing which resulted in a low moisture content. After the 8- and 20-week periods, the gradual decrease in the number of bacteria was observed, which agrees with the results obtained in Colby silt loam (fig. 6).

TABLE IV.—*Influence of magnesium carbonate on the number of bacteria in Plainfield sand*

Treatment.	Number of bacteria in 1 gm. of dry soil.									
	After 1 week.	Relative.	After 2 weeks.	Relative.	After 3 weeks.	Relative.	After 8 weeks.	Relative.	After 20 weeks.	Relative.
None	10,300,000	100	3,500,000	100	5,500,000	100	4,700,000	100	2,450,000	100
One-fourth magnesium carbonate	21,100,000	203	5,100,000	145	10,400,000	190	5,800,000	123	3,560,000	145
One-half magnesium carbonate	13,500,000	131	5,300,000	151	19,300,000	350	10,300,000	219	5,620,000	228
Full magnesium carbonate	73,300,000	711	8,100,000	230	58,300,000	1,060	16,000,000	340	6,530,000	266

The results secured with these two soil types show clearly that magnesium carbonate in certain soils is a potent factor in the reproduction of soil bacteria.

INFLUENCE OF A MIXTURE OF CALCIUM AND MAGNESIUM CARBONATE ON THE NUMBER OF BACTERIA IN SOIL

In every case magnesium carbonate gave a much greater increase in the number of bacteria than did calcium carbonate. Therefore the question which suggests itself is, What effect will a mixture of calcium and magnesium carbonates have on the soil flora?

It was shown by many investigators, principally Loew (*46*) and his associates, that a soil should contain calcium and magnesium in a certain ratio in order to secure the best plant growth. On the other hand, Lipman (*38*) made the following statement:

> In their behavior toward salts, bacteria differ in some respects from both plants and animals and occupy a position by themselves.

From his study of *B. subtilis*, he concluded that no antagonism exists between calcium and magnesium. In a later publication Lipman (*39*) gave an extensive review of the literature bearing on the subject of the

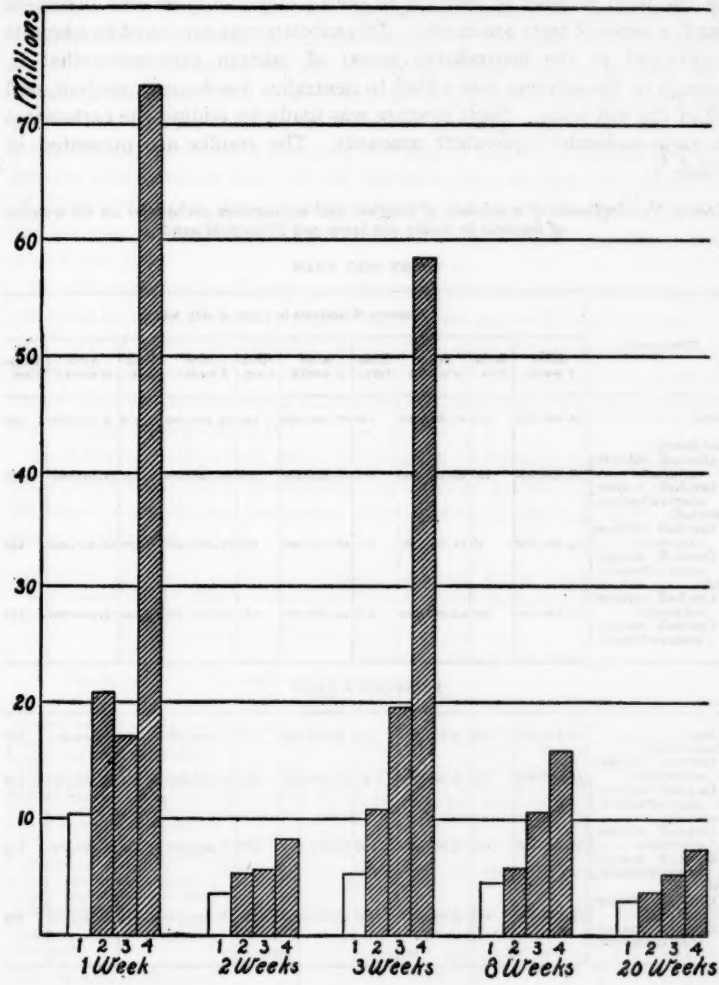

FIG. 6.—Diagram showing the influence of magnesium carbonate on the number of bacteria in Plainfield sand.

1=no treatment. 3=one-half magnesium carbonate.
2=one-fourth magnesium carbonate. 4=full magnesium carbonate.

proper lime-magnesia ratio in soils. He concluded that there is little evidence to support this hypothesis of the lime-magnesia ratio. In accord with Lipman's results, certain investigators (*28*, *29*) showed that various

mixtures of calcium and magnesium carbonates are not favorable to the development of soil bacteria.

To study the effect of a mixture of calcium and magnesium carbonates on the total number of bacteria in both Colby silt loam and Plainfield sand, a series of tests was made. This mixture was employed in amounts equivalent to the neutralizing power of calcium carbonate—that is, enough of the mixture was added to neutralize one-fourth, one-half, and all of the soil acids. Each mixture was made by adding the carbonates in gram-molecular equivalent amounts. The results are presented in Table V.

TABLE V.—*Influence of a mixture of calcium and magnesium carbonates on the number of bacteria in Colby silt loam and Plainfield sand*

COLBY SILT LOAM

Treatment.	Number of bacteria in 1 gm. of dry soil.									
	After 1 week.	Relative.	After 2 weeks.	Relative.	After 3 weeks.	Relative.	After 8 weeks.	Relative.	After 20 weeks.	Relative.
None	19,200,000	100	27,600,000	100	27,500,000	100	14,200,000	100	8,700,000	100
One-fourth: One-half calcium carbonate. One-half magnesium carbonate.	36,500,000	190	29,000,000	105	27,500,000	100	20,700,000	145	15,100,000	173
One-half: One-half calcium carbonate. One-half magnesium carbonate.	14,700,000	76	32,500,000	117	28,600,000	104	17,000,000	120	12,200,000	140
Full: One-half calcium carbonate. One-half magnesium carbonate.	31,800,000	165	45,300,000	163	44,500,000	162	20,000,000	140	13,300,000	152

PLAINFIELD SAND

None	10,300,000	100	3,500,000	100	5,000,000	100	4,700,000	100	2,450,000	100
One-fourth: One-half calcium carbonate. One-half magnesium carbonate.	11,300,000	109	3,200,000	91	11,700,000	234	5,500,000	117	4,250,000	173
One-half: One-half calcium carbonate. One-half magnesium carbonate.	11,800,000	115	3,800,000	109	13,300,000	266	8,400,000	180	3,500,000	143
Full: One-half calcium carbonate. One-half magnesium carbonate.	13,100,000	127	5,000,000	143	18,400,000	370	8,600,000	184	2,620,000	107

It will be seen from the figures of the table that a mixture of calcium and magnesium carbonates increased the number of bacteria in Colby silt loam and Plainfield sand. The most interesting fact ascertained from this test is that magnesium carbonate plus calcium carbonate is less efficient in its effect on the reproduction of bacteria than the equivalent weight of magnesium alone.

INFLUENCE OF CALCIUM CARBONATE, CALCIUM CHLORID, MAGNESIUM CARBONATE, MAGNESIUM CHLORID, DIBASIC MAGNESIUM PHOSPHATE, AND MONOCALCIUM PHOSPHATE ON THE BACTERIA IN SOIL.

COLBY SILT LOAM.—Since the increase in number of bacteria in soil treated with magnesium carbonate seemed far too great to be accounted for by the correction of the soil acidity alone, an experiment was made to study the effect of a neutral salt of magnesium, and a magnesium phosphate, on the number of bacteria in Colby silt loam soil. Two points were considered in planning this test: First, the action of the magnesium and calcium ions on the bacteria; and second, the possibility of the combining of the calcium or magnesium, especially the latter, with the phosphate of the soil, thus liberating the phosphate in a more available form.

It was reported by Truog (*56*) that magnesium phosphate favors the phosphorus assimilation by plants more than any other phosphate. If this be true, then the bacteria (lower plant life) should be favored by a phosphate when in this form. Therefore the question arises, Does the magnesium carbonate when added to soil react with the phosphates to form magnesium phosphate?

In order to make these tests, magnesium carbonate, magnesium chlorid, dibasic magnesium phosphate, also calcium carbonate, calcium chlorid, and monocalcium phosphate were added to the soil alone and in various mixtures.

TABLE VI.—*Influence of calcium carbonate, calcium chlorid, magnesium carbonate, magnesium chlorid, dibasic magnesium phosphate, and monocalcium phosphate on the bacteria of Colby silt loam*

Treatment.	Number of bacteria in 1 gm. of dry soil.							
	After 1 week.	Relative.	After 2 weeks.	Relative.	After 3 weeks.	Relative.	After 8 weeks.	Relative.
None	28,000,000	100	19,000,000	100	23,200,000	100	12,200,000	100
0.1 per cent magnesium chlorid	16,000,000	57	22,100,000	116	14,000,000	60	17,700,000	145
0.1 per cent calcium chlorid	18,200,000	65	17,600,000	92	27,500,000	118	10,500,000	86
Full calcium carbonate	89,100,000	318	37,600,000	197	43,300,000	186	26,200,000	214
Full magnesium carbonate	165,000,000	590	121,100,000	637	200,000,000	862	58,700,000	481
0.1 per cent dibasic magnesium phosphate	30,500,000	108	13,100,000	67	14,000,000	60	15,600,000	127
0.1 per cent monocalcium phosphate	38,000,000	135	9,300,000	47	24,000,000	103	11,600,000	95
0.1 per cent dibasic magnesium phosphate+full calcium carbonate	74,100,000	264	66,100,000	347	33,100,000	141	26,000,000	213
0.1 per cent monocalcium phosphate+full calcium carbonate	38,000,000	135	47,300,000	249	46,200,000	199	31,500,000	258
0.1 per cent dibasic magnesium phosphate+full magnesium carbonate	191,000,000	682	190,000,000	1,000	106,000,000	456	85,500,000	700
0.1 per cent monocalcium phosphate+full magnesium carbonate	223,000,000	800	144,000,000	755	197,000,000	849	78,000,000	639

The effect of magnesium phosphate on the number of bacteria was compared with that of magnesium carbonate, calcium phosphate, and calcium carbonate. If the action of the magnesium and calcium carbonates

on the bacteria is derived from the basic part of these compounds, then the salts of these substances—namely, magnesium and calcium chlorid—should increase the number of bacteria in a similar manner. These salts were added in quantities equivalent to 0.1 per cent of the dry weight of the soil. The data for this experiment are presented in Table VI.

The chlorids of calcium and magnesium failed to increase the number of bacteria in Colby silt loam. Instead, the majority of cases showed a loss, especially with calcium chlorid. Calcium and magnesium carbonates, in agreement with a previous study, increased the number of microorganisms. Magnesium carbonate stimulated the development of the bacteria far greater than did calcium carbonate.

When the dibasic magnesium phosphate and monocalcium phosphate were applied alone there did not seem to be any gain in the number of bacteria. The mixtures of monocalcium phosphate and dibasic magnesium phosphate with magnesium carbonate resulted in all except one period in an increase in the number of bacteria beyond that obtained by the use of magnesium carbonate alone. The mixtures made by adding these same phosphates with calcium carbonate did not result in an increase in the number of bacteria beyond that noted where calcium carbonate was used alone.

From the data of this experiment, which are diagramed in figures 7 and 8, it seems safe to conclude that neither the phosphates nor the chlorids of magnesium and calcium alone caused a marked multiplication of the bacteria in Colby silt loam.

MIAMI SILT LOAM.—A similar test of these different compounds was made with Miami silt loam, a neutral soil. The chlorids of magnesium and calcium were eliminated in this experiment. Since the effect of a carbonate on the number of bacteria in Miami silt loam soil was not known, the carbonates were added in amounts equal to the one-half and full calcium carbonate requirement of Colby silt loam. The data for this experiment are presented in Table VII.

TABLE VII.—*Influence of calcium carbonate, magnesium carbonate, dibasic magnesium phosphate, and monocalcium phosphate on the number of bacteria in Miami silt loam*

Treatment.	Number of bacteria in 1 gm. of dry soil.					
	After 1 week.	Relative.	After 2 weeks.	Relative.	After 3 weeks.	Relative.
None	10,000,000	100	18,500,000	100	9,600,000	100
One-half calcium carbonate	10,300,000	103	18,500,000	100	13,800,000	143
Full calcium carbonate	13,800,000	138	23,700,000	128	15,100,000	157
One-half magnesium carbonate	17,000,000	170	25,400,000	137	15,500,000	161
Full magnesium carbonate	27,000,000	270	52,400,000	283	21,600,000	225
0.1 per cent monocalcium phosphate	10,600,000	106	20,400,000	110	12,300,000	128
0.1 per cent dibasic magnesium phosphate	15,700,000	157	27,000,000	146	14,300,000	150
0.1 per cent monocalcium phosphate + full calcium carbonate	12,400,000	124	24,000,000	130	13,500,000	140
0.1 per cent dibasic magnesium phosphate + full calcium carbonate	11,100,000	111	20,000,000	108	14,600,000	152
0.1 per cent monocalcium phosphate + full magnesium carbonate	20,000,000	200	35,000,000	189	19,000,000	198
0.1 per cent dibasic magnesium phosphate + full magnesium carbonate	21,600,000	216	43,800,000	236	11,700,000	121

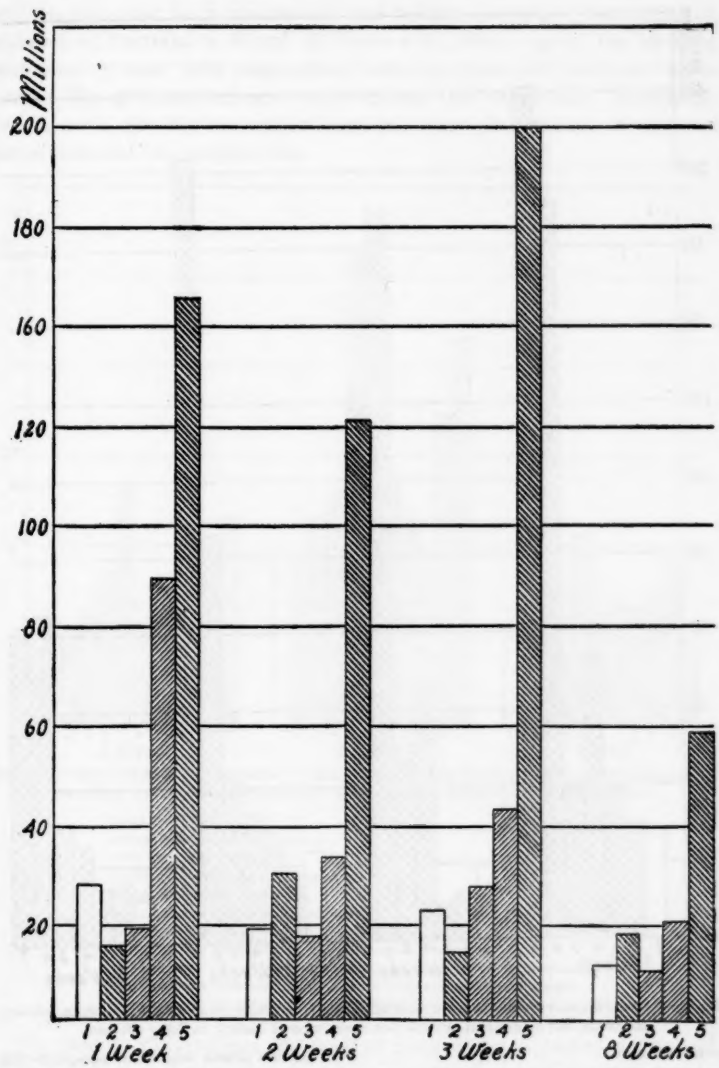

FIG. 7.—Diagram showing the influence of the carbonates and chlorids of magnesium and calcium on the number of bacteria in Colby silt loam soil.

1=no treatment.
2=0.1 per cent magnesium chlorid.
3=0.1 per cent calcium chlorid.
4=full calcium carbonate.
5=full magnesium carbonate.

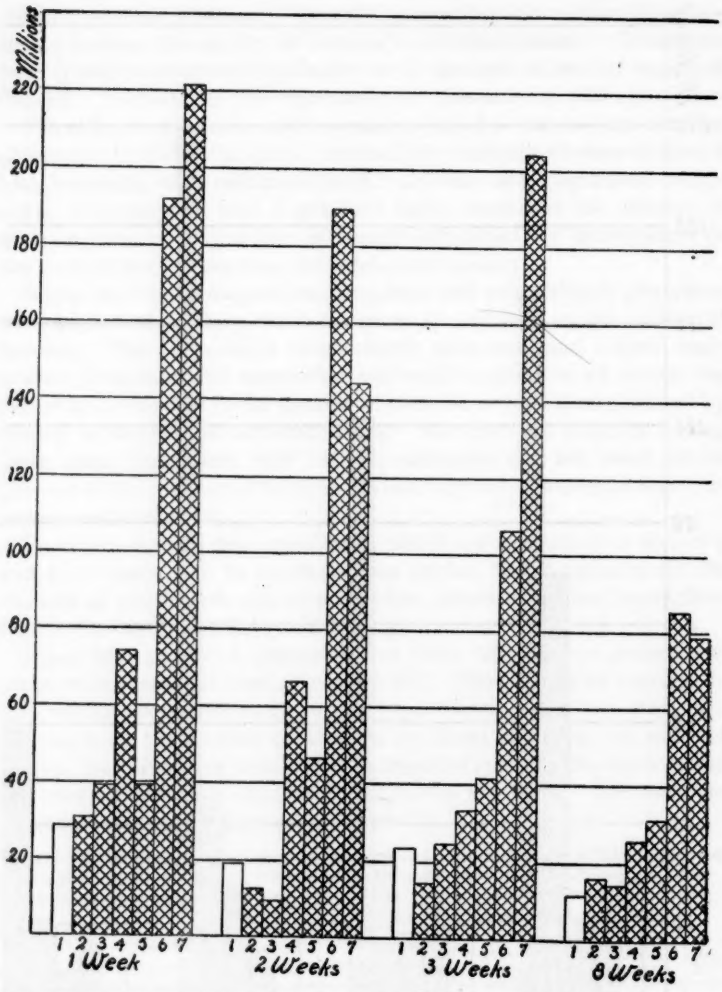

FIG. 8.—Diagram showing the influence of dibasic magnesium phosphate, monocalcium phosphate, calcium carbonate, and magnesium carbonate on the number of bacteria in Colby silt loam.

1=no treatment.
2=0.1 per cent dibasic magnesium phosphate.
3=0.1 per cent monocalcium phosphate.
4=0.1 per cent dibasic magnesium phosphate+full calcium carbonate.
5=0.1 per cent monocalcium phosphate+full calcium carbonate.
6=0.1 per cent dibasic magnesium phosphate+full magnesium carbonate.
7=0.1 per cent monocalcium phosphate+full magnesium carbonate.

From the results which are given in Table VII and from figure 9 it will be seen that both magnesium and calcium carbonate increased the number of bacteria in Miami silt loam soil. Here, again, the increase was much greater with magnesium carbonate than with calcium carbonate. The greatest increase was obtained two weeks after treatment. In all cases, the heavier applications increased the number of bacteria more than did the smaller ones.

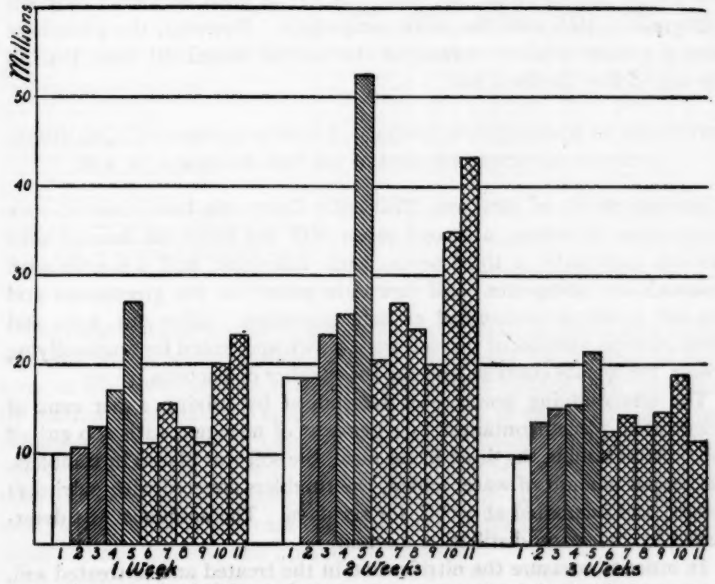

FIG. 9.—Diagram showing the influence of calcium carbonate, magnesium carbonate, dibasic magnesium phosphate, and monocalcium phosphate on the number of bacteria in Miami silt loam.

1=no treatment.
2=one-half calcium carbonate.
3=full calcium carbonate.
4=one-half magnesium carbonate.
5=full magnesium carbonate.
6=0.1 per cent monocalcium phosphate.
7=0.1 per cent dibasic magnesium phosphate.
8=0.1 per cent monocalcium phosphate+full calcium carbonate.
9=0.1 per cent dibasic magnesium phosphate+full calcium carbonate.
10=0.1 per cent monocalcium phosphate +full magnesium carbonate.
11=0.1 per cent dibasic magnesium phosphate+full magnesium carbonate.

The results differentiate themselves from those obtained with acid Colby silt loam soil since the phosphates increased the number of bacteria in the neutral Miami silt loam. It was expected that these phosphates would have a more beneficial effect in neutral than in acid soil, since it is probable that the phosphate was hydrolyzed, thus adding more acid to the soil. In every test magnesium phosphate gave a larger increase in number of bacteria than was obtained by the use of calcium phos-

phate. The dibasic magnesium phosphate increased the number of bacteria to about the same degree as an application of one-half magnesium carbonate. A combination of each phosphate with each carbonate did not prove beneficial in augmenting the number of bacteria in Miami silt loam beyond that caused by each carbonate when used alone. It is evident from the foregoing data, as shown in figure 9, that the relative increase of the number of bacteria from the use of carbonates of calcium and magnesium in Miami silt loam was not so great as when Colby silt loam was treated with the same compounds. However, the phosphate gave a greater relative increase in the neutral Miami silt loam than in the acid Colby silt-loam soil.

INFLUENCE OF MAGNESIUM CARBONATE, CALCIUM CARBONATE, LIMESTONE, AND MONOCALCIUM PHOSPHATE ON THE BACTERIA IN SOIL

Another series of jars was filled with Colby silt loam treated with magnesium carbonate; a second series with the same soil treated with calcium carbonate; a third series with limestone, and a fourth with monocalcium phosphate. All jars were placed in the greenhouse and the soil moisture maintained at half saturation. After one, two, and three months samples of this soil were drawn and tested for ammonifying power, for nitrate content, and for the number of bacteria.

The ammonifying power was determined by mixing 2 per cent of dried blood, which contained 13.4 per cent of nitrogen, with 100 gm. of soil. After adding the dried blood meal, the soil was placed in tumblers, the proper amount of water added, the tumblers covered with petri-dish covers, and incubated at 27° C. for six days. The ammonia was determined by the steam-distillation method.

In order to measure the nitrification in the treated and untreated soil, samples were taken from the jars and the nitrate content determined immediately. This was simply a study of nitrate accumulation in the soil; no nitrogenous substance was added. Nitrates were determined by the phenol-disulphonic acid method. The data for the experiments with Colby silt loam and Plainfield sand are shown in Tables VIII to XI, inclusive.

From the data in Table VIII it will be seen that after one month the increase in the number of bacteria in Colby soil, in conformity with previous tests, was greatest with the magnesium-carbonate treatment. The increase was very marked, about five times greater than that derived from the use of calcium carbonate or limestone. The favorable effect of the magnesium carbonate was noted after both the 2- and 3-month periods. In most cases the calcium carbonate and limestone increased the number of bacteria, notably after two months. The monocalcium phosphate apparently exerted no effect in increasing the

number of bacteria, since it gave no increase except when used in combination with calcium carbonate, and then the increase was no greater than that obtained with calcium carbonate alone.

TABLE VIII.—*Influence of calcium carbonate, magnesium carbonate, limestone, and monocalcium phosphate on the number of bacteria in Colby silt loam*

Treatment.	Number of bacteria in 1 gm. of dry soil.					
	After 1 month.	Relative.	After 2 months.	Relative.	After 3 months.	Relative.
None	14,000,000	100	10,700,000	100	8,000,000	100
One-fourth calcium carbonate	14,370,000	102	13,400,000	125	8,800,000	110
One-half calcium carbonate	12,200,000	87	17,000,000	158	7,100,000	88
Full calcium carbonate	14,370,000	102	14,800,000	138	8,700,000	108
One-fourth limestone	12,200,000	87	17,600,000	164	10,300,000	128
One-half limestone	14,370,000	102	11,200,000	104	8,700,000	108
Full limestone	13,340,000	95	14,300,000	133	10,700,000	133
One-fourth magnesium carbonate	15,180,000	108	11,900,000	111	10,900,000	136
One-half magnesium carbonate	26,000,000	185	25,700,000	240	13,200,000	165
Full magnesium carbonate	101,400,000	724	43,700,000	408	33,500,000	418
0.1 per cent monocalcium phosphate	15,000,000	107	10,700,000	100	8,300,000	103
0.1 per cent monocalcium phosphate + one-fourth calcium carbonate	18,100,000	130	10,800,000	101	7,600,000	95
0.1 per cent monocalcium phosphate + full calcium carbonate	11,380,000	80	13,600,000	127	10,300,000	128

The data in Table IX are in agreement with those obtained with Colby silt loam—that is, magnesium carbonate increased the number of bacteria in Plainfield sand to a considerable extent beyond that produced by calcium carbonate or limestone. The effect of the magnesium carbonate on the number of bacteria was most noticeable one month after the treatment was applied. In general, limestone proved inferior to calcium carbonate in stimulating the number of bacteria in Plainfield sand, except that in one case the monocalcium phosphate when applied alone did not increase the number of bacteria.

TABLE IX.—*Influence of calcium carbonate, magnesium carbonate, limestone, and monocalcium phosphate on the number of bacteria in Plainfield sand*

Treatment.	Number of bacteria in 1 gm. of dry soil.					
	After 1 month.	Relative.	After 2 months.	Relative.	After 3 months.	Relative.
None	6,800,000	100	6,550,000	100	2,100,000	100
One-fourth calcium carbonate	6,220,000	91	5,440,000	83	2,450,000	116
One-half calcium carbonate	6,100,000	90	7,800,000	119	3,200,000	152
Full calcium carbonate	8,770,000	128	8,440,000	128	2,900,000	138
One-fourth limestone	5,600,000	82	5,900,000	90	1,780,000	84
One-half limestone	9,180,000	135	4,900,000	74	2,550,000	121
Full limestone	7,700,000	113	7,660,000	116	2,550,000	121
One-fourth magnesium carbonate	7,240,000	164	5,400,000	82	3,000,000	133
One-half magnesium carbonate	12,640,000	185	7,300,000	111	5,000,000	238
Full magnesium carbonate	44,800,000	658	28,500,000	435	4,800,000	228
0.1 per cent monocalcium phosphate	4,380,000	64	3,000,000	45	2,340,000	111
0.1 per cent monocalcium phosphate + one-fourth calcium carbonate	7,550,000	111	4,670,000	71	3,230,000	154
0.1 per cent monocalcium phosphate + full calcium carbonate	6,620,000	97	4,500,000	68	2,700,000	128

FORMATION OF AMMONIA

The ammonia determinations were made at the end of two and three months. It will be seen from the data in Table X that all treatments accelerated the formation of ammonia from dried blood. After two months monocalcium phosphate plus calcium carbonate showed the greatest effect, although the phosphate-alone treatment gave nearly as good results. Of the carbonates, calcium carbonate appeared to have the greatest effect in increasing the ammonifying power, an effect nearly as great as that obtained from the phosphate applications. Magnesium carbonate exerted a less effect than did calcium carbonate, and limestone had even a less effect than was obtained with magnesium carbonate. After three months the phosphate still retained a lead in stimulating ammonification. Limestone caused a greater increase in ammonia-producing power than did calcium carbonate, while calcium carbonate gave a greater increase than was obtained with magnesium carbonate. From the data on Colby soil it is shown that the increase in ammonifying power is not always parallel with the increase in the total number of organisms.

TABLE X.—*Influence of calcium carbonate, magnesium carbonate, limestone, and monocalcium phosphate on the ammonification of dried blood in Colby silt loam and Plainfield sand*

Treatment.	Ammonia nitrogen in 100 gm. of dry soil.							
	Colby silt loam.				Plainfield sand.			
	After 2 months.	Increase.	After 3 months.	Increase.	After 2 months.	Increase.	After 3 months.	Increase.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
None	37.7	.....	51.7	.....	49.0	.....	55.1	.....
One-fourth calcium carbonate	39.0	+ 1.3	55.5	+ 3.8	47.7	− 1.3	52.2	− 2.9
One-half calcium carbonate	.....	.....	61.6	+ 9.9	40.1	− 8.9	47.0	− 8.1
Full calcium carbonate	53.0	+15.3	62.3	+10.6	40.1	− 8.9	53.1	− 2.0
One-fourth limestone	45.7	+ 8.0	61.6	+ 9.9	51.8	+ 2.8	51.0	− 4.1
One-half limestone	40.6	+ 2.9	64.5	+12.8	38.7	−10.3	51.3	− 3.8
Full limestone	44.2	+ 6.5	69.7	+18.0	37.7	−11.3	73.8	+18.7
One-fourth magnesium carbonate	49.0	+11.3	58.1	+ 6.4	40.3	− 8.7	46.7	− 8.4
One-half magnesium carbonate	51.6	+13.9	53.7	+ 2.0	39.9	− 9.1	47.5	− 7.6
Full magnesium carbonate	57.1	+19.4	52.5	+ 1.8	35.2	−13.8	47.0	− 8.0
0.1 per cent monocalcium phosphate	61.0	+23.3	63.1	+11.4	56.7	+ 7.7	75.3	+20.2
0.1 per cent monocalcium phosphate+one-fourth calcium carbonate	57.5	+19.8	78.0	+26.3	50.5	+ 1.5	73.3	+18.2
0.1 per cent monocalcium phosphate+full calcium carbonate	72.5	+34.8	80.6	+28.9	48.5	− 0.5	65.7	+10.6

The data in Table X for Plainfield sand showed very different results from those obtained with Colby silt loam. After two months a decrease in ammonifying power was noted, except in the case of the one-fourth limestone treatment. The phosphate when applied alone gave the greatest increase, but where calcium carbonate was added in combination with the phosphate, the formation of ammonia was not so great.

With an increase of calcium carbonate there was a decrease in ammonia production. This decrease was shown with all carbonates, magnesium carbonate causing the greatest decrease.

After three months the same order was held by these compounds in stimulating ammonia formation. At this time the full limestone had a greater effect than the smaller limestone applications; the effect was almost as great as that due to the phosphate treatment. It is evident from the results obtained in both Colby silt loam and Plainfield sand, that the greatest accumulation of ammonia (six days' incubation) does not occur where the largest increase in the number of bacteria was obtained. Where the greatest number of bacteria developed, it seems probable that the greatest amount of ammonia should be formed. Since the substances which gave the highest number of bacteria also neutralized the soil acids, it is probable that the ammonia partly escaped through volatilization. Because of the open texture of the Plainfield sand, more ammonia escaped from this soil than from the Colby silt loam.

TABLE XI.—*Influence of calcium carbonate, magnesium carbonate, limestone, and monocalcium phosphate on nitrate accumulation in Colby silt loam and Plainfield sand*

Treatment.	Nitrate nitrogen accumulated in 100 gm. of dry soil.					
	Colby silt loam.					
	After 1 month.	Increase.	After 2 months.	Increase.	After 3 months.	Increase.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
None	5.86	.....	6.05	.....	6.62	.....
One-fourth calcium carbonate	7.23	1.37	6.94	0.89	8.56	1.94
One-half calcium carbonate	6.10	.24	7.56	1.51	9.37	2.75
Full calcium carbonate	6.25	.37	8.18	2.13	12.00	5.38
One-fourth limestone	5.53	— .33	6.05	.00	7.25	.63
One-half limestone	5.86	.00	6.40	.35	7.25	.63
Full limestone	6.51	.65	6.42	.37	8.56	.94
One-fourth magnesium carbonate	6.58	.72	6.75	.70	9.43	2.81
One-half magnesium carbonate	8.13	2.27	8.44	2.39	12.00	5.38
Full magnesium carbonate	13.28	7.42	13.00	6.95	15.00	8.38
0.1 per cent monocalcium phosphate	5.63	— .23	6.05	.00	7.25	.63
0.1 per cent monocalcium phosphate + one-fourth calcium carbonate	6.10	.24	6.42	.37	9.37	2.75
0.1 per cent monocalcium phosphate + full calcium carbonate	6.73	.87	8.18	2.13	11.25	4.63

Treatment.	Nitrate nitrogen accumulated in 100 gm. of dry soil.					
	Plainfield sand.					
	After 1 month.	Increase.	After 2 months.	Increase.	After 3 months.	Increase.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
None	1.74	.....	1.58	.....	1.33	.....
One-fourth calcium carbonate	2.42	0.68	1.85	0.27	1.67	0.34
One-half calcium carbonate	2.35	.61	2.31	.73	2.55	1.22
Full calcium carbonate	2.20	.46	2.22	.64	2.92	1.59
One-fourth limestone	2.20	.46	1.80	.22	1.73	.40
One-half limestone	1.87	.13	1.67	.09	2.00	.67
Full limestone	1.97	.23	1.87	.29	2.80	1.47
One-fourth magnesium carbonate	2.10	.36	1.24	— .34	1.50	.17
One-half magnesium carbonate	2.20	.46	2.22	.64	2.70	1.37
Full magnesium carbonate	2.34	.60	2.78	1.20	4.62	3.29
0.1 per cent monocalcium phosphate	2.12	.38	1.85	.27	3.08	1.75
0.1 per cent monocalcium phosphate + one-fourth calcium carbonate	2.35	.61	2.41	.83	2.20	.87
0.1 per cent monocalcium phosphate + full calcium carbonate	2.80	.06	1.80	.22	3.08	1.75

ACCUMULATION OF NITRATES

From the data presented in Table XI it will be seen that nitrates accumulated faster in both the treated Colby silt loam and Plainfield sand than in the untreated. In both soils the magnesium carbonate benefited nitrification more than did the other carbonates. Next in order to magnesium carbonate was calcium carbonate, and lastly, lime-

stone, in increasing the nitrate content of Colby silt loam and Plainfield sand; the heavier applications gave the highest nitrate accumulation. In the case of the magnesium carbonate with Colby silt loam soil, the increased accumulation reached as high as 8 mgm. The increase in nitrate in the Plainfield sand ran parallel with that in the Colby silt loam where similarly treated, though the accumulation in the sand was much smaller, since this soil is low in organic matter.

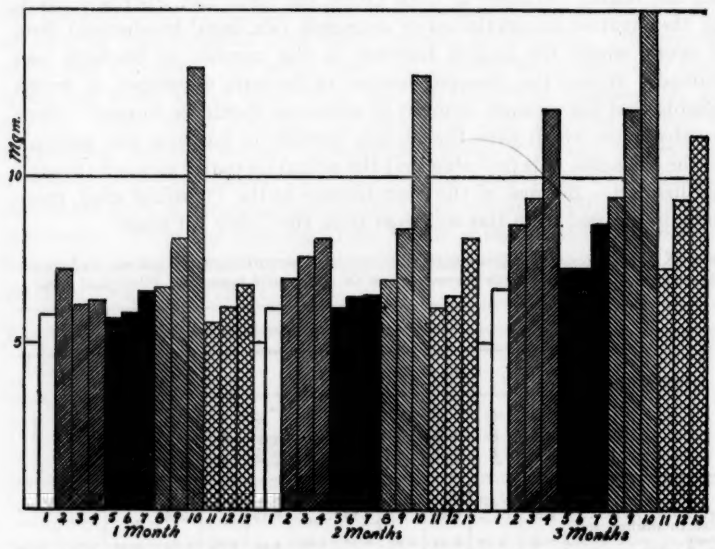

FIG. 10.—Diagram showing the influence of calcium carbonate, magnesium carbonate, limestone, and monocalcium phosphate on nitrate accumulation of Colby silt loam.

1=no treatment.
2=one-fourth calcium carbonate.
3=one-half calcium carbonate.
4=full calcium carbonate.
5=one-fourth limestone.
6=one-half limestone.
7=full limestone.
8=one-fourth magnesium carbonate.
9=one-half magnesium carbonate.
10=full magnesium carbonate.
11=0.1 per cent monocalcium phosphate.
12=0.1 per cent monocalcium phosphate+one-fourth calcium carbonate.
13=0.1 per cent monocalcium phosphate+full calcium carbonate.

The monocalcium phosphate apparently did not favor nitrification in the Colby soil, while in the sand this substance proved beneficial. A combination of the phosphate and calcium carbonate did not increase nitrification beyond that obtained from the use of calcium carbonate alone. The effect of these substances on the reproduction of soil organisms and on the formation of nitrates was similar. A review of the entire data is shown graphically in figure 10.

INFLUENCE OF CALCIUM CARBONATE, MAGNESIUM CARBONATE, LIMESTONE, AND MONOCALCIUM PHOSPHATE ON THE NITRIFICATION OF GELATIN IN SOIL.

In order to study the effect of calcium carbonate, magnesium carbonate, limestone, and monocalcium phosphate on nitrification in Colby and in Plainfield sand to which nitrogenous material was added, a series of tests was made. After the soils had been treated with the forenamed substances separately and allowed to incubate for three months, 100-gm. samples were drawn and to each portion 23.6 mgm. of gelatin were added. The soil was placed in tumblers, which were covered with glass, and incubated for six weeks at 27° C. At the end of this time the nitrate content was determined. The results of this experiment are given in Table XII.

TABLE XII.—*Influence of calcium carbonate, magnesium carbonate, limestone, and monocalcium phosphate on the nitrification of gelatin in Colby silt loam and in Plainfield sand*

Treatment.	Nitrate nitrogen in 100 gm. of dry soil.			
	Colby silt loam.		Plainfield sand.	
	After 6 weeks.	Increase.	After 6 weeks.	Increase.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
None	20.87	.....	9.06	.....
One-fourth calcium carbonate	22.31	1.44	11.70	2.64
One-half calcium carbonate	25.00	4.13	10.57	1.51
Full calcium carbonate	28.43	7.56	11.12	2.06
One-fourth limestone	20.87	.00	11.70	2.64
One-half limestone	23.12	2.25	11.70	2.64
Full limestone	25.00	4.13	11.12	2.06
One-fourth magnesium carbonate	23.12	2.25	11.12	2.06
One-half magnesium carbonate	26.12	5.25	13.09	4.03
Full magnesium carbonate	25.00	4.13	12.24	3.18
0.1 per cent monocalcium phosphate	22.31	1.44	9.06	.00
0.1 per cent monocalcium phosphate+one-fourth calcium carbonate	23.12	2.25	13.09	4.03
0.1 per cent monocalcium phosphate+full calcium carbonate	28.43	7.56	12.24	3.18

In reviewing Table XII it will be seen that with the exception of one-fourth limestone treatment in Colby loam, and phosphate alone in Plainfield sand, there was an increase in nitrification in the treated soils. The increase in most cases was very slight, especially in the sand, where only about half of the nitrogen of gelatin apparently was nitrified, while in the Colby loam the greater part of the nitrogen of gelatin was nitrified. A comparison of the amount of nitrate formed in the soil to which magnesium or calcium carbonate was added, both with and without the addition of gelatin (Table XI), showed a relatively greater nitrate formation in the soils to which no organic nitrogen was added. These results were to be expected, since it is likely that some of the ammonia formed from the gelatin in the neutral or partly neutral soil escaped. On the other hand, the combined effects of the gelatin and the carbonates increased the multiplication of bacteria beyond that of the soil treated with gelatin alone. The great gain in the number of bacteria is no doubt

followed by an increase in assimilation of nitrates. These results agree with those obtained by Fred and Graul (*20*).

In the Colby silt loam the full calcium carbonate gave the best results while magnesium carbonate and limestone rank in the order named. The magnesium carbonate when added to the soil with gelatin did not give as good results as calcium carbonate. This difference may be due to the fact that the large increase in the number of bacteria caused by the magnesium carbonate treatment favored a greater assimilation of nitrate by the microorganisms. Plainfield sand apparently gave better results where the one-fourth or one-half neutralization was obtained. Very probably the sand releases more ammonia when neutral than does the Colby silt loam. In both soils the phosphate benefited nitrification but slightly.

INFLUENCE OF CALCIUM CARBONATE, MAGNESIUM CARBONATE, LIMESTONE, AND MONOCALCIUM PHOSPHATE ON NITROGEN FIXATION IN SOIL

An effort was made to study the influence which carbonate and phosphate treatments would have on the independent nitrogen-fixing organism, *Bacillus azotobacter chroococcum*. Three months after treatment, Colby and Plainfield soils were sampled and the soil placed in large soup plates, 1 per cent of mannit was added to each plate of soil, which was then inoculated with a culture of *B. azotobacter*. After incubating for four weeks in the greenhouse, the soils were dried and ground to a very fine powder in a ball mill. Total nitrogen determinations (Kjeldahl method modified to include nitrates) were then made. Duplicate determinations were made from each plate and an average of these taken. The results for this experiment are given in Table XIII.

TABLE XIII.—*Influence of calcium carbonate, magnesium carbonate, limestone, and monocalcium phosphate on nitrogen fixation in Colby silt loam and Plainfield sand treated with mannit and inoculated with Bacillus azotobacter*

Treatment.	Nitrogen in 100 gm. of dry soil.							
	Colby silt loam.				Plainfield sand.			
	After 4 weeks.		Average.	Increase.	After 4 weeks.		Average.	Increase.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
None	275.0	275.0	275.0	.....	63.0	61.0	62.0	.....
One-fourth calcium carbonate	277.0	276.0	276.5	+1.5	59.0	59.0	59.0	−3.0
One-half calcium carbonate	273.0	275.0	274.0	−1.0	61.0	61.0	61.0	−1.0
Full calcium carbonate	276.0	276.0	276.0	+1.0	64.0	62.0	63.0	+1.0
One-fourth limestone	275.0	275.0	275.0	.0	61.0	59.0	60.0	−2.0
One-half limestone	273.0	275.0	274.0	−1.0	61.0	63.0	62.0	.0
Full limestone	278.0	279.0	278.5	+3.5	62.0	64.0	63.0	+1.0
One-fourth magnesium carbonate	277.0	276.0	276.5	+1.5	59.0	59.0	59.0	−3.0
One-half magnesium carbonate	278.0	276.0	277.0	+2.0	62.0	62.0	62.0	.0
Full magnesium carbonate	275.0	275.0	275.0	.0	63.0	63.0	63.0	+1.0
0.1 per cent monocalcium phosphate	274.0	276.0	275.0	.0	61.0	61.0	61.0	−1.0
0.1 per cent monocalcium phosphate + one-fourth calcium carbonate	279.0	282.0	280.5	+5.5	61.0	59.0	60.0	−2.0
0.1 per cent monocalcium phosphate + full calcium carbonate	283.0	277.0	280.0	+5.0	65.0	65.0	65.0	+3.0

From the data presented in Table XIII it appears that there was no decided gain in total nitrogen in the Plainfield sand, and only a small gain in Colby silt loam. It is difficult to explain why no gain, and in some cases a loss, of nitrogen was found. The loss may have been due to a disturbance in the balance of the flora of these soils. Since the mannit which was added to the soil, and the neutralization of some of the soil acids by the basic substances caused a great increase in the number of bacteria, it is possible that a part of the nitrate nitrogen was set free as elemental nitrogen. However, a gain in total nitrogen is shown in the Colby silt loam soil due to the carbonate and limestone treatment.

INFLUENCE OF CALCIUM CARBONATE, MAGNESIUM CARBONATE, AND LIMESTONE ON ORGANISMS IN PURE CULTURE

An effort was made to determine the influence of calcium carbonate, magnesium carbonate, and limestone on pure cultures of bacteria in sterilized soil. In order to simplify the work, only Colby soil was employed.

It was observed that when this soil is heated for a long period at a high temperature, there is a reduction in soil acidity. Other investigators have reported similar results in acid soils. Conner (*10*) showed a decrease in acidity when soil was heated to complete dryness. He suggested that possibly the acid silicates were hydrolyzed with the formation of a base. On heating soil to 140° C. for one hour, Sharp and Hoagland (*55*) noted a decrease in acidity. On the other hand, Schreiner and Lathrop (*54*) increased the acidity in an acid-reacting soil by heating the soil for three hours at 30 pounds' pressure, which in all probability changed the organic matter to such an extent as to increase the acidity. The work of Kelley and McGeorge (*30*) and Darbishire and Russell (*11*) showed a change in the soil consituents on heating, especially in the solid inorganic constituents, which were made more soluble.

In order to overcome as much as possible this reduction in acidity, various methods of sterilization were tried. Both dry and moist soils were sterilized in Erlenmeyer flasks, which were heated in steam under a pressure of 15 pounds for three hours. On testing this heated soil for its degree of acidity by the Truog zinc-sulphid test, the dry soil showed practically no change in its acid content, while the moist soil, one-half and full water-saturated, showed a considerable decrease. The very wet soil after sterilization contained less acid than that which received one half as much water. From the evidence it seems safe to conclude that the silicates of this soil are partially hydrolyzed with the formation of bases.

Further tests were conducted with dry soil. It was dried for 24 hours at about 45° C. and placed in flasks; these were then plugged and sterilized for three hours at 15 pounds' pressure. After sterilization, the soil received water sufficient to bring it to half saturation. This was done to determine if hydrolysis took place by adding water after the soil

had been sterilized. At the end of four days tests were made on the dry and on the watered soil. The acidity of both appeared unchanged.

From the results of the foregoing experiments it appears that dry Colby soil can be sterilized and sterile water added until the soil is half saturated, without interfering seriously with the original reaction of the soil. Therefore this method of sterilizing soil was adopted for all of the pure-culture work.

INFLUENCE OF CALCIUM CARBONATE, MAGNESIUM CARBONATE, AND LIMESTONE ON THE AMMONIFICATION OF BLOOD MEAL BY PURE CULTURES OF BACTERIA IN SOIL

COLBY SILT LOAM.—One-hundred-gm. portions of dry soil were each treated with calcium carbonate, with magnesium carbonate, and with limestone separately. To the soil of the entire series 2 per cent of dried-blood meal was applied. The treated soil was placed in 300-c. c. Erlenmeyer flasks and sterilized as previously stated. Sterilized water was then added to bring the soil to half saturation. The sterilized soil was then inoculated with water suspensions of *Bacillus tumescens* and *B. subtilis*. The flasks were incubated at 27° C. for seven days and at the end of this period the ammonia in each flask was determined. In Table XIV are recorded the complete data for this experiment.

TABLE XIV.—*Influence of calcium carbonate, magnesium carbonate, and limestone on the ammonification of dried blood with pure cultures in Colby silt loam*

Treatment.	Ammonia nitrogen in 100 gm. of dry soil.			
	Bacillus tumescens.		*Bacillus subtilis.*	
	After 7 days.	Increase.	After 7 days.	Increase.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
None	11.3	.....	11.0	.....
One-fourth calcium carbonate	15.5	4.2	.....	.....
One-half calcium carbonate	16.4	5.1	16.0	5.0
Full calcium carbonate	16.4	5.1	16.0	5.0
One-fourth limestone	11.2	−0.1	11.7	0.7
One-half limestone	14.6	3.3	13.0	2.0
Full limestone	14.8	3.5	13.9	2.9
One-fourth magnesium carbonate	15.7	4.4	12.5	1.5
One-half magnesium carbonate	16.5	5.2	17.6	6.6
Full magnesium carbonate	17.1	5.8	21.0	10.0

The data in Table XIV show clearly that the ammonification of dried blood by *Bacillus tumescens* or *B. subtilis* was increased when the sterilized soil was treated with calcium carbonate or with magnesium carbonate or with limestone. Magnesium carbonate gave better results than did calcium carbonate, while the latter gave better results than did the limestone. In every case full treatment with any of the compounds gave the largest increase of ammonia. This is in agreement with results obtained in unsterilized Colby silt loam two months after treatment with the limestone or calcium carbonate.

INFLUENCE OF CALCIUM CARBONATE, MAGNESIUM CARBONATE, AND LIMESTONE ON BACILLUS AZOTOBACTER IN STERILIZED SOIL

COLBY SILT LOAM.—Dry soil in 200-gm. portions was treated with calcium carbonate, with magnesium carbonate, and limestone, and placed in 500-c. c. Erlenmeyer flasks. To each flask was added 1 per cent of mannit by weight of dry soil. The flasks were plugged, sterilized, and sterile water was added to bring the soil to the proper moisture content. Inoculations of *Bacillus azotobacter* in pure culture were then made by adding a 1-c. c. suspension of the organisms to each flask. The entire set of flasks was incubated at 27° C., and after one, two, and three weeks plate counts, using mannit agar, were made. The results for this experiment are recorded in Table XV.

TABLE XV.—*Influence of calcium carbonate, magnesium carbonate, and limestone on Bacillus azotobacter in Colby silt loam treated with mannit*

Treatment.	Number of bacteria in 1 gm. of dry soil.		
	After 1 week.	After 2 weeks.	After 3 weeks.
None	<1,000	<1,000	<1,000
One-fourth calcium carbonate	6,602,000	4,170,000	2,440,000
One-half calcium carbonate	2,200,000	12,500,000	32,300,000
Full calcium carbonate	8,400,000	11,250,000	45,500,000
One-fourth limestone	<1,000	<1,000	<1,000
One-half limestone	<1,000	<1,000	<1,000
Full limestone	<1,000	850,000	26,000,000
One-fourth magnesium carbonate	26,200,000	3,620,000	10,350,000
One-half magnesium carbonate	5,150,000	47,000,000	268,000,000
Full magnesium carbonate	30,800,000	477,000,000	355,000,000

The results of this experiment showed the striking effect of magnesium carbonate on the multiplication of *Bacillus azotobacter* cells in Colby silt loam soil. Calcium carbonate caused an increase in the number of these organisms, but the increase was not as great as that obtained with magnesium carbonate. With limestone the increase was very small. This great gain with magnesium carbonate was obtained where amounts equal to full neutralization were applied. After the third week the one-half neutralization by magnesium carbonate also gave a large increase, while two weeks after treatment the greatest gain was shown.

INFLUENCE OF LARGE APPLICATIONS OF MAGNESIUM CARBONATE ON BACILLUS AZOTOBACTER IN STERILE SOIL

COLBY SILT LOAM.—Because magnesium carbonate when applied to give neutralization, increased the number of *Bacillus azotobacter* in sterilized Colby silt loam soil to a great extent, a further test was made to determine if heavier applications, enough added to give a distinct alkaline reaction, would continue to increase the reproduction of *Bacillus azotobacter*. These applications were made by adding magnesium carbonate sufficient to make 1¼, 1½, and double neutralization.

The data for this test, presented in Table XVI, showed that 1½ magnesium—carbonate treatment after one week gave a greater increase in the number of *Bacillus azotobacter* cells than was obtained by the 1¼ application; after two and three weeks, the 1¼ treatment gave a greater increase than was obtained by the 1½ treatment. In every case the heaviest application of magnesium carbonate caused less increase in the number of these organisms than did the lighter treatments. However, as compared with the control, the heavy application also gave a great increase.

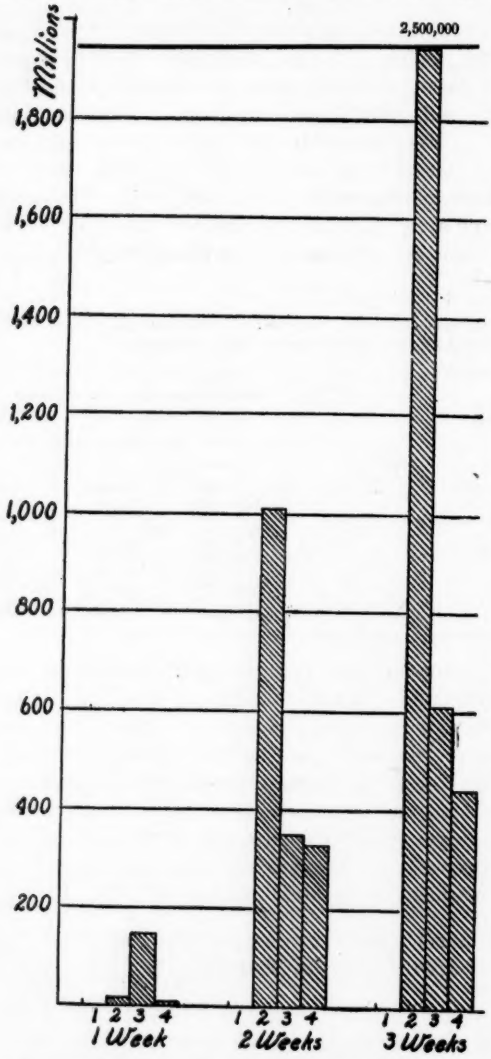

FIG. 11.—Diagram showing the influence of large applications of magnesium carbonate on *Bacillus azotobacter* in sterile Colby silt loam.

1=no treatment. 3=1½ magnesium carbonate.
2=1¼ magnesium carbonate. 4=double magnesium carbonate.

In comparing the data of Table XVI with those of Table XV, it will be seen that 1¼ and in most cases 1½ magnesium-carbonate treatment caused a greater increase in the number of *Bacillus azotobacter* organisms in sterilized Colby silt loam than the full treatment. It is difficult to explain why such an enormous increase in the number of these organisms was obtained when more than enough magnesium carbonate was added to neutralize the soil acids. From the data in Table XVI, one is led to believe that this great multiplication in *Bacillus azotobacter* cells was due

to something besides the correction of the soil acidity. The entire data in Table XVI are presented graphically in figure 11.

TABLE XVI.—*Influence of large applications of magnesium carbonate on Bacillus azotobacter in Colby silt loam treated with mannit*

Treatment.	Number of bacteria in 1 gm. of dry soil.		
	After 1 week.	After 2 weeks.	After 3 weeks.
None	<1,000	<1,000	<1,000
One and one-fourth magnesium carbonate	13,500,000	1,030,000,000	2,500,000,000
One and one-half magnesium carbonate	147,500,000	357,000,000	605,000,000
Double magnesium carbonate	10,300,000	338,000,000	481,000,000

INFLUENCE OF MAGNESIUM CARBONATE ON BACILLUS AZOTOBACTER IN STERILE NEUTRAL SOIL

MIAMI SILT LOAM.—Since magnesium carbonate increased the number of *Bacillus azotobacter* cells in an acid soil when more than enough of the carbonate was added to neutralize the acidity, an experiment was planned to determine the effect this compound would have on *Bacillus azotobacter* in a neutral soil. The soil selected for this work was Miami silt loam, previously described. Magnesium carbonate, along with 1 per cent mannit, was added in one-fourth, one-half, and full neutralization based on the acidity of Colby silt loam. The data for this test are tabulated in Table XVII.

TABLE XVII.—*Influence of magnesium carbonate on Bacillus azotobacter in sterile Miami silt loam treated with mannit*

Treatment.	Number of bacteria in 1 gm. of dry soil.					
	After 1 week.	Relative.	After 2 weeks	Relative.	After 3 weeks.	Relative.
None	3,020,000	100	37,500,000	100	51,000,000	100
One-fourth magnesium carbonate	2,870,000	95	52,500,000	140	127,000,000	249
One-half magnesium carbonate	44,000,000	1,456	11,200,000	30	137,000,000	269
Full magnesium carbonate	67,700,000	2,241	26,200,000	70	392,000,000	768

In studying the data in this table, it will be seen that in Miami silt loam, a neutral soil, where magnesium carbonate was applied in an amount great enough to neutralize all the active acidity in Colby soil, there was a great increase in the number of *Bacillus azotobacter* cells. At this time no explanation can be offered to account for the drop in number after two weeks where one-half and full magnesium carbonate were applied, since the other counts, made after one and three weeks with the one-half and full application gave a decided increase. The count made after the 3-week period showed the greatest increase. From the results of Table XVII it is clear that magnesium carbonate plays some rôle in stimulating the *B. azotobacter* organism other than that of neutralization.

INFLUENCE OF CALCIUM CARBONATE, MAGNESIUM CARBONATE, AND LIMESTONE ON BACILLUS RADICICOLA (ALFALFA) IN STERILE SOIL

COLBY SILT LOAM.—This experiment was planned to measure the effect of calcium carbonate, magnesium carbonate, and limestone on the reproduction of *Bacillus radicicola* in sterilized Colby silt loam soil. The alfalfa strain, which is supposed to be sensitive to acidity, was selected for this work. The soil was treated with 1 per cent of mannit, and the magnesium carbonate was added. Two-hundred-gm. portions of the treated soil were placed in 500-c.c. Erlenmeyer flasks. The flasks were then plugged and sterilized according to the method used in the preceding experiments. Inoculations were made by introducing a 1-c.c. suspension of the organisms.

From the data presented in Table XVIII it will be seen that calcium and magnesium carbonates increased greatly the number of alfalfa organisms in this soil. The magnesium carbonate after one week did not increase the number of these organisms to any greater extent than did calcium carbonate. The smaller amounts were surprisingly effective, though the increase was not as great as that obtained with larger amounts. Limestone increased the number of these organisms to a very slight extent.

TABLE XVIII.—*Influence of calcium carbonate, magnesium carbonate, and limestone on Bacillus radicicola (alfalfa) in sterile Colby silt loam treated with mannit*

Treatment.	Number of bacteria in 1 gm. of dry soil.		
	After 1 week.	After 2 weeks.	After 3 weeks.
None	13,500	1,200	1,200
One-fourth calcium carbonate	340,000,000	1,350,000,000	1,170,000,000
One-half calcium carbonate	1,310,000,000	1,650,000,000	1,600,000,000
Full calcium carbonate	1,750,000,000	1,620,000,000	1,870,000,000
One-fourth limestone	101,000	100,000	6,200
One-half limestone	227,000	5,000	50,000
Full limestone	2,450,000	4,170,000	240,000,000
One-fourth magnesium carbonate	87,000,000	1,212,000,000	1,540,000,000
One-half magnesium carbonate	4,100,000,000	1,112,000,000	1,770,000,000
Full magnesium carbonate	4,900,000,000	600,000,000	925,000,000

INFLUENCE OF CALCIUM AND MAGNESIUM CARBONATES ON BACILLUS RADICICOLA (LUPINE) IN STERILIZED SOIL

COLBY SILT LOAM.—Since the alfalfa strain of *Bacillus radicicola* was greatly benefited by the carbonates of magnesium and calcium, it seemed desirable to see what effect a similar treatment would have on the lupine strain of *B. radicicola*, which is frequently termed an acid-resistant organism. Limestone was eliminated in this experiment; otherwise the procedure for this test was similar to that in the preceding experiment. The results of this test are tabulated in Table XIX.

TABLE XIX.—*Influence of calcium carbonate and magnesium carbonate on Bacillus radicicola (lupine) in sterile Colby silt loam treated with mannit*

Treatment.	Number of bacteria in 1 gm. of dry soil.		
	After 1 week.	After 2 weeks.	After 3 weeks.
None	112,000	30,000	60,000
One-fourth calcium carbonate	875,000,000	3,770,000,000	5,530,000,000
One-half calcium carbonate	1,300,000,000	3,630,000,000	3,820,000,000
Full calcium carbonate	1,837,000,000	2,760,000,000	4,200,000,000
One-fourth magnesium carbonate	886,000,000	4,025,000,000	125,000,000
One-half magnesium carbonate	950,000,000	4,837,000,000	5,000,000,000
Full magnesium carbonate	1,750,000,000	8,850,000,000	9,170,000,000

From the data given in Table XIX it is evident that the lupine strain of *Bacillus radicicola* was benefited to as great an extent as the alfalfa strain when Colby soil was treated with magnesium or calcium carbonate. After the second and third weeks the counts showed that magnesium carbonate gave better results than did calcium carbonate. The untreated soil gave a slightly higher number than was obtained with the alfalfa strain under similar conditions. This difference may be due to the fact that the lupine strain is slightly tolerant to an acid reaction.

Here, as in the tests made with unsterilized soil, the smaller applications stimulated multiplication to a greater extent, in proportion to the amounts applied, than did the larger treatments. However, the maximum gain in number of bacteria was obtained with full magnesium-carbonate treatment. From the data obtained in this and the preceding experiment it is clearly evident that either magnesium or calcium carbonate (the magnesium to a somewhat greater degree than the calcium) greatly increased the number of legume bacteria, alfalfa and lupine strains, in an acid soil.

CONCLUSIONS

From a general review of the results of the experiments just described it is clearly shown that carbonates of calcium and magnesium when applied to acid Colby silt loam, acid Plainfield sand, and neutral Miami silt loam increase the number of bacteria. A greater increase was obtained in the acid soils than in the neutral Miami silt loam. These carbonates increased the number of bacteria in both sterilized and unsterilized soil. The sterilized soil was inoculated with an organism in pure culture. Not only was the number of bacteria in the soils increased by applications of magnesium and calcium carbonates, but an increase in activity of the various groups of bacteria was shown.

The form of carbonate which gave the best results was magnesium carbonate. There was an enormous increase in the number of bacteria produced by the magnesium carbonate over that of calcium carbonate. Ground dolomitic limestone did not prove quite as efficient as calcium

carbonate in increasing the number of soil bacteria. This variation can possibly be explained by the difference in solubility of these compounds. It was shown (*50*) that magnesium carbonate is more soluble in carbonated water than is calcium carbonate, and that calcium carbonate is more soluble than limestone. Dolomitic limestone, however, is less soluble than the nondolomitic. This order of solubility is in agreement with the order in which the bacteria of the soil responded to treatment with these different compounds.

It does not seem correct to say that the entire influence which these compounds had on the soil bacteria was due to the neutralization of the soil acids. When neutral soil was treated with magnesium carbonate or with calcium carbonate, the number of bacteria was increased, especially in the case of magnesium carbonate. Both in neutral and in acid soil, the latter doubly neutralized, magnesium carbonate increased the number of *Bacillus azotobacter*. All this would indicate that carbonates either serve in part as a stimulant or effect an indirect action on other compounds which are in turn rendered more soluble.

Since the magnesium and calcium carbonates increased the number of bacteria in acid soil when added in small amounts, and since application in acid soil of these compounds gave better results than when applied to neutral soil, it appears that the greater part of this influence on the bacteria was due to neutralization.

It may have been that the magnesium carbonate when added to the soil was partially converted into magnesium phosphate. Truog (*56*) pointed out the fact that for the amount of phosphate used, magnesium phosphate increased the phosphorus content of plants more than did other forms of phosphates. If this be the case, and the formation of magnesium phosphate takes place in soil when magnesium carbonate is applied, then magnesium chlorid and magnesium phosphate should give an increase in the number of bacteria. The results from the magnesium-chlorid treatment did not prove beneficial, however, even in so small amounts; the chlorin radical may have been toxic. Magnesium phosphate did not prove favorable to the reproduction of bacteria in acid soil but did in neutral soil. In the latter soil, the effect of magnesium phosphate was more beneficial than the effect of calcium phosphate. From this evidence it appears that magnesium phosphate in a soil favorable for the development of bacteria is a stimulant to the growth of bacteria. This action of magnesium phosphate on bacteria in soil may in part account for the enormous influence which magnesium carbonate exerts in increasing the number of bacteria in both neutral and acid soils.

From all the data obtained in the various experiments performed, magnesium carbonate appears to play an important part in the development of soil bacteria, much more so than does calcium carbonate. Magnesium phosphate, when applied to neutral soil, caused an increase

in the number of bacteria. In the neutral soil magnesium phosphate proved superior to calcium phosphate in stimulating the bacteria; in acid soil neither compound appeared to benefit the soil flora.

SUMMARY

(1) The number of bacteria in acid Colby silt loam and acid Plainfield sand is increased by the applications of calcium carbonate, magnesium carbonate, or limestone.

(2) Magnesium carbonate increases the number to a much greater extent than does either calcium carbonate or limestone.

(3) Monocalcium phosphate and dibasic magnesium phosphate slightly increase the total number of bacteria in neutral soil.

(4) Nitrification is benefited by limestone, calcium-carbonate, and magnesium-carbonate treatment. Magnesium carbonate in soil to which no nitrogenous substance was added favors nitrate accumulation more than does either calcium carbonate or limestone. The phosphates increase the accumulation of nitrate nitrogen to a very small extent. When gelatin was applied to the soil, magnesium carbonate did not benefit nitrification any more than calcium carbonate or limestone.

(5) Ammonification in Colby soil is benefited by all three forms of the carbonates, while in Plainfield sand a decrease in ammonia is shown. Monocalcium phosphate increases ammonification in both soils.

(6) Pure cultures of *Bacillus tumescens* and *B. subtilis* ammonify blood meal better when sterile Colby soil is treated with any one of the three forms of carbonates.

(7) A culture of *B. azotobacter* in the two soils treated with the carbonates and mannit fails to show an increase in total nitrogen in the sand and only a slight gain in the Colby soil.

(8) Pure cultures of *B. radicicola*, of both alfalfa and lupine strains, and *B. azotobacter* are greatly benefited when inoculated into sterile Colby soil previously treated with magnesium or calcium carbonate. Limestone barely increases the number of *B. azotobacter* in Colby soil. In neutral and acid soils treated with magnesium carbonate until the soils were strongly alkaline, *B. azotobacter* is greatly increased in munber over that of the untreated.

(9) From the data considered as a whole, magnesium carbonate is superior to calcium carbonate or limestone in stimulating the reproduction of bacteria in Colby silt loam and Plainfield soils. In general, the smaller applications of either compound give better results than do the heavier applications.

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HUMUS IN MULCHED BASINS, RELATION OF HUMUS CONTENT TO ORANGE PRODUCTION, AND EFFECT OF MULCHES ON ORANGE PRODUCTION

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INTRODUCTION

The formation of humus in a soil and its conservation are points that are usually given much weight in discussions of soil fertility. The free formation of humus in a soil from organic matter added to it is usually held to indicate that the soil its biologically active, and proper biological activity in a soil is conceded to be necessary under the usual agricultural practices. Hence, whatever the absolute value of soil humus may be as a factor in crop production, it is ordinarily conceded, other factors being equal, that a soil which readily forms humus when organic matter is added is superior to one that does not.

Previous work (*1*)[1] by this Office in southern California has shown that the mottling of Citrus leaves varied inversely with the humus content in the soil. In other work by the same writers (*2*) it was shown that on certain soil types in the general region of Riverside, California, the use of the mulched-basin system in Citrus groves improved the tree conditions more than the usual system of cultivation and furrow irrigation, and that different green-manure substances when used as a mulch affected tree growth and fruit setting in varying degrees.

It is therefore of practical value to obtain information about the rate of humus formation from various organic substances when employed either as a mulch or when worked into the soil, and to ascertain, if possible, whether the increase in humus is correlated with increase in tree growth and fruit setting.

This work reports the study of humus formation in mulched basins in Citrus groves, and the effect of different mulching materials on fruit production and tree growth. The work was done at Riverside.

The term "humus" as used in this paper is the brown-colored organic matter extracted from a soil by boiling it for two minutes in a 7.5 per cent sodium-hydrate solution, after the removal of calcium from the soil with 1 per cent hydrochloric acid.

The percentage of humus was determined colorimetrically by comparing the intensity of the color of the soil extract thus obtained with the intensity of a standard humus solution prepared from the humus ex-

[1] Reference is made by number (italic) to "Literature cited," pp. 517-518

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tracted from peat. In the great number of humus determinations made by the Office of Biophysical Investigations in the Citrus areas studied in California not a single humus extract obtained has been off-color, when compared with the standard solutions prepared for comparison.

Gortner (*4*) obtained a black soil pigment from the soils he studied by extracting them successively with 4 per cent sodium hydroxid, 1 per cent hydrochloric acid, 4 per cent sodium hydroxid followed by water. The soil residue obtained from the last sodium-hydroxid extraction, when shaken with water in quantity, yielded the black pigment.

Soils from various Citrus areas studied in this work did not produce black pigment in appreciable amounts when they had been extracted with hydrochloric acid to the absence of calcium, boiled for two minutes in a 7.5 per cent sodium-hydroxid solution, and washed with hot dilute sodium-hydroxid solution on the filter till the "humus color" had been removed. It might be noted that a boiling 5 per cent solution of sodium hydroxid did not remove all the color from the soils, and that a 10 per cent solution removed no more than a 7.5 per cent solution.

Following Gortner's procedure, black pigments were obtained from some of the soils investigated in the Citrus areas of southern California.

It should be noted that the colorimetric reading should be made as soon as the humus extract is obtained, as the color partially fades out on standing.

As no evidence was obtained to indicate that any coloring matter was left in the soil after the extraction with boiling sodium hydroxid of the strength noted, and as the color tint was always the same as that of the standard used, it seems that this rapid method for humus determination is reliable for comparative studies, especially when used with soils in the same general area.

Another objection to the colorimetric method is that undecomposed organic matter, like dry alfalfa, sweet clover, etc., produces a color when treated in the manner just described for making humus determinations. This objection is also brought out by Gortner (*4*). This matter was looked into when the method was worked out. When dry hay susbtances were extracted with hydrochloric acid to the absence of calcium, the chlorin washed out, and the residue boiled for several minutes with a 7.5 per cent solution of sodium hydroxid, the organic materials yielded a yellow-colored solution entirely different in color from the brown to black humus color.

A number of readings of these organic extracts gave a density of color corresponding to about 0.0012 per cent humus in soil, if it is assumed that 1 per cent of the substances was to be added to the soil. This is about the working error in making humus determinations by the method used, as will be seen from Table II, and such errors could have no influence on comparative results in the kind of study here reported. Indeed there could never have been much of this off-color in the extracts, as a

uniform tint was always obtained from the soils studied in making humus extracts from them. The straw color produced by the extraction of soil with 1 to 2 per cent of undecomposed organic matter added was found to be so thoroughly obscured by the humus color of the soil that its presence did not interfere with the colorimetric reading.

It is well recognized that when hay material of many kinds is digested with hot hydrochloric acid and extracted with ammonia, a solution is obtained which in appearance and color can not be distinguished from a humus extract from a soil.

Much of the literature relating to humus is discussed by Schreiner and Shorey (*10*).

HUMUS FORMATION IN MULCHED BASINS

EXPERIMENT I.—Mulched basins, 15 feet in diameter, were installed in an orange grove near Riverside, Cal., in March, 1915. The soil is a red clay loam, derived from granite, which underlies the soil at a depth of 3 to 4 feet. One half of the basins were mulched with about 180 pounds of alfalfa (*Medicago sativa*) each and the other half with about 20 cubic feet of good cow manure each. One half of the basins in each mulching series received 200 pounds of lime dust each. This lime was a by-product from the flues of a neighboring cement plant. It was composed of about 45 per cent of calcium carbonate, about 20 per cent of calcium hydrate, about 10 per cent of calcium sulphate, about 1 per cent of potash, and a little phosphoric acid. The balance was mostly silicious material.

During August of the same year another row of orange trees in the same grove was basined and mulched with alfalfa. These basins were smaller than those just referred to, and less mulch was used. Part of these basins received about 100 pounds each of ground lime rock analyzing about 90 per cent calcium carbonate. The rest of the basins in the row were unlimed.

All surface organic matter was carefully removed, and soil samples were taken to a depth of 3 feet in these basins, usually from three basins in each experiment, the samples being separated into foot sections. Corresponding foot samples from the three basins were composited, and each composite sample was analyzed in duplicate. The percentage reported in the following tables are the averages to a depth of 3 feet. Usually the samples were collected just before irrigation.

Table I shows the average percentage of humus from time to time to a depth of 3 feet of soil in the basins under the various treatments during a period of from 12 to 17 months.

The low humus percentage is due in part to the removal of several inches of the surface soil in constructing the basins, which suggests incidentally that in making basins as little surface soil as possible should be removed.

TABLE I.—*Percentage of humus to a depth of 3 feet in mulched basins in orange groves on clay loam soil. Experiment I*

Date.	Basin treatment and percentage of humus.					
	Large basins installed in March.				Small basins installed in August.	
	Alfalfa alone.	Manure alone.	Alfalfa and lime.	Manure and lime.	Alfalfa alone.	Alfalfa and lime.
1915.						
Mar. 31	0.075	0.118	0.113	0.129	.....	.....
Aug. 13	.073	.130	.106	.112	.....	.....
Sept. 13	.075	.136	.147	.139	0.166	0.165
Oct. 12	.084	.153	.155	.160	.114	.092
Nov. 11	.098	.186	.185	.151	.118	.108
Nov. 20	.187	.188	.150	.151	.....	.....
Dec. 11	.198	.180	.188	.156	.121	.096
Average for 1915	.113	.156	.149	.143	.130	.115
1916.						
Mar. 17	.113	.201	.180	.179	.171	.115
Apr. 17	.111	.246	.287	.213	.257	.259
May 15	.076	.133	.181	.141	.133	.107
June 16	.112	.231	.288	.220	.165	.139
July 13	.081	.295	.272	.207	.117	.111
Aug. 7	.185	.292	.215	.227	.212	.175
Average for 1916	.113	.233	.237	.198	.176	.151
Ratio 1916 to 1915	.000	1.49	1.59	1.38	1.35	1.31

It will be observed that the determinations show a fluctuation in the percentage of humus from time to time. This is doubtless due partly to the difficulty of getting uniform soil samples. This factor, however, does not afford a complete explanation, because the same kind of fluctuation was noted when determinations were made on soils kept in pots in the laboratory, where better control conditions obtained and where leaching was avoided. Neither can the fluctuations be due entirely to the working error in making humus determinations, since these errors are of smaller magnitude than the variations in the humus content of the soils.

The following duplicate determinations made on March 31, 1915, the averages of which are given on the first line in Table I, illustrate the working error in making humus determinations by the method employed (Table II).

Heinze (*6*) states that after humus has been formed other bacteria, such as Azotobacter, commence to decompose it. McBeth (*9*) lays stress on the fact that the cellulose destroying organisms in the soil break down the organic matter in the soils with the formation of humus, and that the nitrifying organisms break the humus up into still simpler compounds through their nitrifying activities.

TABLE II.—*Working error in making humus determinations*

Soil No.	Humus.	Soil No.	Humus.	Soil No.	Humus.
	Per cent.		*Per cent.*		*Per cent.*
677	0.358	676	0.227	685	0.284
677	.358	676	.227	685	.284
678	.041	677	.051	686	.081
678	.039	677	.050	686	.081
679	.045	678	.023	687	.050
679	.046	678	.022	687	.050
670	.250	682	.192	688	.284
670	.250	682	.200	688	.284
671	.030	683	.056	689	.074
671	.028	683	.051	689	.070
672	.048	684	.022	690	.043
672	.046	684	.021	690	.042

From these considerations it might therefore be expected that the amount of humus in the soil in the basins would be subject to fluctuation, the amount present at any one time depending upon the ascendency of one or the other of these important groups of soil organisms.

The fluctuation in the percentage of humus makes it impracticable to attempt to determine the rate at which humus was formed between specific dates. It will be seen in Table I that there is frequently a decrease in the percentage of humus from one period to another, especially during the second year, a point that might possibly apply to determinations made by Gortner (5). He added silk, wool, flour, and alfalfa meal to soils, kept the materials in earthenware jars, and at the end of a year made humus determinations. In most instances he found less humus at the end of the year than he found in the treated soils when the experiment was started. It would have been of much interest if these determinations had been made frequently during the year. None of the losses of humus reported by him are greater than was sometimes found in a month's time in humification studies carried out under laboratory control in this Office, to be reported later.

A comparison of the amount of humus in the basins from year to year is made by averaging the percentage found during each of the years 1915 and 1916, as shown in Table I. At the bottom of the table will be found the ratio of the average percentage of humus in 1916 to that in 1915.

EXPERIMENT II.—In April, 1915, a block of orange trees in another grove on sandy loam soil was basined. The basins were about 12 feet in diameter, and the treatments were the same as in Experiment I, except that less lime was used.

The results of the humus determinations are given in Table III, which shows the fluctuation in the amount of humus from time to time. The ratio of the average percentage of humus in the basins in 1916 to that in 1915 is given in the bottom line in the table.

TABLE III.—*Percentage of humus to depth of 3 feet in mulched basins in orange groves on sandy loam soil. Experiment II*

Date.	Basin treatment and percentage of humus.			
	Alfalfa alone.	Manure alone.	Alfalfa and lime.	Manure and lime.
1915.				
May 3	0.169	0.159	0.214	0.196
Aug. 9	.128	.121	.126	.164
Sept. 4	.129	.146	.187	.189
Oct. 4	.230	.218	.283	.260
Oct. 30	.203	.209	.255	.307
Nov. 30	.185	.159	.181	.212
Average for 1915	.174	.169	.208	.221
1916.				
Mar. 17	.251	.241	.301	.286
Apr. 17	.227	.220	.296	.338
May 15	.129	.180	.163	.180
June 16	.216	.213	.309	.....
July 13	.195	.167	.244	.248
Aug. 7	.175	.296	.243	.309
Average for 1916	.199	.220	.259	.272
Ratio, 1916 to 1915	1.14	1.30	1.24	1.23

EXPERIMENT III.—In October, 1915, a block of 60 trees in another orange grove of heavy sandy loam soil was basined and mulched. The basins were about 6 by 20 feet. The mulching materials used, the quantity of each, and the percentage of humus in the soil are given in Table IV. The percentages are averages to a depth of 3 feet. The amount of bean straw used was not enough to cover the soil, and so did not make an effective mulch. Some of these basins received in addition to the mulching material, 100 pounds ground lime rock, analyzing about 90 per cent calcium carbonate.

TABLE IV.—*Average percentage of humus to a depth of 3 feet in mulched basins in an orange grove on heavy sandy loam. Experiment III*

Basin treatment.	Percentage of humus.		Humus ratio—1916 to 1915.	Quantity of mulch and lime per basin.
	Oct. 13, 1915.	June 27, 1916.		
Alfalfa alone	0.223	0.316	1.41	125 pounds.
Alfalfa and lime	.160	.185	1.16	100 pounds of lime.
Bean straw alone	.243	.220	.91	70 pounds.
Bean straw and lime	.241	.240	1.00	100 pounds of lime.
Manure alone	.264	.226	.86	20 cubic feet.
Manure and lime	.244	.163	.67	100 pounds of lime.
Barley hay alone	.319	.247	.78	125 pounds.
Barley hay and lime	.235	.194	.83	100 pounds of lime.
Sweet clover alone	.254	.161	.63	125 pounds.
Sweet clover and lime	.237	.215	.91	100 pounds of lime.
Bur clover alone	.279	.203	.73	125 pounds.
Pine shavings alone	.266	.279	1.05	125 pounds.
Pine shavings and lime	.265	.206	.78	100 pounds of lime.

The basins with alfalfa as the mulch were the only ones which increased in percentage of humus during the interval given. Other experiments have shown that bean straw readily humifies. It is possible that if this had been applied in as large a quantity as the other substances, these basins would have increased in humus also. The lime was not consistent in its effect on the amount of humus formed.

It is to be noted, however, that the percentage of humus as found in the summer, after the application of the mulches in the preceding fall, does not necessarily indicate that in most of these basins the average amount of humus had actually decreased. As shown in Tables I and III, there are periodical fluctuations in the humus content, and the second set of determinations shown in Table IV may have been made at a time when the amount of humus was comparatively small. The figures in Table IV probably give an indication of the relative humifying activity in the basins. Each percentage given is an average of about six determinations.

EXPERIMENT IV.—A series of basins was installed in a lemon grove near Corona, Cal., in October, 1915. The soil is sandy loam in texture interspersed freely with gravel so that samples below 3 feet can not be taken with the ordinary soil tube.

The mulching materials used were alfalfa and manure and, in addition to the organic mulch, some of the basins received other artificial materials—viz, blood, phosphate, tankage, sulphur, bone meal, and lime. In addition to the basined rows, several control rows were retained, and were manured, irrigated, and cultivated in the usual way. The amount of manure applied to the trees in the control rows was the same as applied in the basins.

Soil samples were collected after the basins were made, on October 20, 1915, and another set was taken on June 29, 1916. The detailed humus determinations made on the latter date did not indicate that any of the artificial substances added to the mulches produced any definite influence on the humus content. The average percentage of humus to a depth of 3 feet in the alfalfa basins, manure basins, and in the control rows is given in Table V. Each percentage figure given in the table is made up of an average of about 21 determinations, each determination representing a composite of at least three samples.

TABLE V.—*Average percentage of humus to a depth of 3 feet in unmulched soil and in mulched basins in a lemon grove on light sandy loam soil. Experiment IV*

Soil treatment.	Percentage of humus.		Ratio 1916 to 1915.
	Oct. 20, 1915.	June 29, 1916.	
Basins with alfalfa mulch	0.360	0.306	0.85
Basins with manure mulch	.345	.361	1.05
Furrow irrigation and surface cultivation; manured	.551	.380	.60

The low initial percentage of humus in the basins is partly due to the fact that 5 or 6 inches of the surface soil were removed in making the basins, owing to sloping ground.

The percentage of humus in the manured basins in 1916 was about the same as in 1915, while in the alfalfa basins it was less. The decrease in the humus content in the manured soil furrow-irrigated and surface-cultivated is very appreciable, indicating that this system did not conserve the humus as well as the basin system.

The grove in which this experiment was conducted was deteriorating along with other groves in the neighborhood when the basins were installed. The whole grove was in better condition in October, 1916, but there was no apparent difference in the appearance of or the amount of fruit on the trees basined as compared with trees not basined. The mulch-basin system in experiments conducted by this office has always produced a quicker response on trees on clay loam soil than on trees on sandy loam or light sandy loam soil.

The deterioration of the grove above mentioned was evidently due to bad soil conditions, which quite likely affected the bacterial flora in such a way as to make it less efficient in converting the alfalfa into humus.

In most cases presented above the percentage increase in humus during a period of from 9 to 17 months was greater in the basins on the clay loam soil than in those on the sandy loam soil, with both manure and alfalfa as the mulch. On the whole, the manured basins gained slightly more in humus content on both types of soil than the alfalfa-mulched basins. No correction has been made for the amount of humus added with the manure. This material contained about 5 per cent of humus when applied, part of which undoubtedly found its way into the surface soil when the basins were irrigated. The percentage increase of humus in the manured basins therefore does not necessarily indicate that the manure had been humified to a greater extent than alfalfa. Data to be presented in another paper would indicate that the increase is likely due to the humus added with the manure.

In all cases where lime was added to manure in basins the increase in humus was somewhat less than when manure alone was used. In most cases the addition of lime to alfalfa in basins produced slightly more humus than when alfalfa was added alone. Frear and Hess (*3*) found that field plots receiving manure and lime contained less active humus than unmanured plots.

The amount of alfalfa or manure necessary to form a unit amount of humus can not be stated with exactness from the data available. In Experiments I and II the average increase in humus in the basins in 1916 over the average amount present in 1915 would indicate that 1 part of humus was formed from about 10 to 12 parts of alfalfa, and 1 part of humus from about 26 parts of manure. These calculations agree fairly

well with results stated by others (7, *p. 128–129*), but are necessarily only estimates.

The data obtained in the humus studies in mulched basins did not indicate that there was any very appreciable accumulation of humus in the lower soil depths due to leaching from the surface foot of soil. The detailed data showed that most of the change in humus content took place in the surface foot.

RELATION OF HUMUS CONTENT OF THE SOIL TO ORANGE PRODUCTION

EXPERIMENT I.—Picking records of the earlier basined trees in Experiment I were obtained from the company on whose grove the experiment was conducted, and are given in Table VI.

TABLE VI.—*Effect of different mulching materials on orange production. Experiment I. Picked May, 1917*

Basin treatment.	Average number of boxes of oranges per tree.			1917 yield corrected on basis of previous yields (boxes per tree).	Row No.	Tree numbers in row.
	1914	1915	1917			
Alfalfa alone	1.49	0.71	3.31	4.07	3	1 to 8
Alfalfa and lime	1.19	.58	3.61	6.69	3	26 to 33
Manure alone	1.78	1.00	2.16	2.56	3	9 to 15
Manure and lime	1.32	1.02	1.81	2.55	3	16 to 25

This appreciable difference in fruit production of trees mulched with alfalfa and manure, respectively, does not correlate with the humus content in the soil under the respective mulches, as may be seen from Table I. The manured basins average a slightly higher percentage of humus than the basins mulched with alfalfa.

It will also be noticed in Table VI that the trees basined and mulched with manure in March, 1915, had previously produced slightly more fruit than the trees mulched with alfalfa.

When the oranges were picked in the spring of 1917, the color of the oranges on the trees mulched with alfalfa was discinctly more golden than the color of the oranges on the trees mulched with manure.

EXPERIMENT II.—Individual tree picking records had not been kept of the trees in the grove in which this experiment was conducted. Individual tree picking records were obtained in the spring of 1917 from the company on whose grove the experiment was conducted. In this experiment a row of trees was left unbasined and was manured, furrow-irrigated, and cultivated in the usual manner. The amount of manure per tree was the same as that put into the basins.

The fruit production of the trees is given in Table VII. The number of trees used in each set of mulched trees was from 13 to 15.

TABLE VII.—*Influence on orange production of different mulches in basins and by furrow irrigation and cultivation. Experiment II. Picked June, 1917*

Soil treatment.	Average number of boxes per tree.	Average for each organic substance with and without lime.
Basins with alfalfa mulch alone	5.3	4.8
Basins with alfalfa mulch and lime	4.5	
Basins with manure mulch alone	4.3	4.0
Basins with manure mulch and lime	3.9	
Cultivated with manure alone, disked	2.9	2.5
Cultivated with manure and lime	2.3	

It is not known that the trees used in this experiment differed in yields previous to the installation of this experiment. However, as no individual tree picking records had been kept previous to the experiment, the comparative yields shown in Table VII do not carry the force they would if they could have been compared with the previous performance of the same trees. In comparing the fruit production in experiments I and III, it seems safe to infer that the results obtained in Experiment II were indicative of the effect of the different organic materials used.

It will be noticed in Table III that the percentage of humus in the manured basins was slightly higher than that in the basins mulched with alfalfa, a result agreeing with that obtained in Experiment I.

No appreciable difference in the color of the fruit from the trees differently treated was discernible.

EXPERIMENT III.—The block of 60 orange trees used in this experiment had been used in a study of individual tree performance by the Office of Horticultural and Pomological Investigations, Bureau of Plant Industry. Individual tree records for the previous six years had been obtained, and these were kindly furnished by the Riverside officials of that Office. The picking records of the 1916–1917 crop are given in Table VIII, together with the basin treatments. The basins were installed and mulched in October, 1915.

No effect of the lime on fruit production could be definitely determined; hence, the yields of all the trees mulched with the same organic matter have been averaged, including both limed and unlimed trees.

The alfalfa-mulched trees produced more fruit than the manure-mulched trees, which result agrees with the results obtained in Experiments I and II. It does not appear from Table VIII that any legume whatever is superior to a nonlegume or to manure. The basined trees mulched with sweet clover and bur clover did not produce as much fruit as those mulched with alfalfa, bean straw, manure, or barley hay.

The color of the oranges on the trees mulched with alfalfa and bean straw was distinctly more golden than the color of the oranges on the trees mulched with the other organic substances. The foliage on the bean-straw and alfalfa-mulched trees was considerably denser and greener than that on the other trees.

TABLE VIII.—*Effect of different mulching materials in basins on orange production. Experiment III. Picked May, 1917*

Basin treatment.	Yield of oranges corrected on basis of previous performance of trees.		
	Pounds per tree.	Oranges per tree.	Average weight per orange.
			Pounds.
Alfalfa hay	308	881	0.350
Bean straw	289	744	.389
Manure	261	611	.427
Barley hay	202	497	.407
Sweet clover hay	217	584	.372
Bur clover hay	216	530	.408
Pine shavings	163	402	.406

On comparing the results of the humus determinations shown in Table IV with the fruit production shown in Table VIII it is evident that the percentage of humus in the soil does not correlate with the fruit production. There is, however, a correlation between the fruit production and the ratio of the humus content in 1916 to that in 1915, but such correlation is not evident in Experiments I and II.

Closer examination of the picking records in Experiment III showed that the bean-mulched trees produced more first-class fruit of the more desirable sizes than the trees mulched with any of the other substances; the alfalfa-mulched trees were second in this respect; manure third; and barley fourth; while the trees mulched with sweet clover and pine shavings produced the least number of fruits of the first quality.

It may be that some toxic substance is formed in the decomposition of the pine shavings which might account for the poor condition of the trees and for the small yield obtained from them; also the shavings probably had a deleterious influence on nitrification. Redwood boxes for use in germinating Citrus seedlings proved unsuitable, because, when the root tips came in contact with the wood, they promptly died.

Oranges from the trees in Experiments I, II, and III were analyzed, the analyses being made by the branch office of the Citrus By-products Laboratory in Los Angeles. The analyses did not bring out any consistent differences in the fruits from trees receiving different fertilizer treatments.

There was a consistent difference in the specific gravity of the oranges from the limed trees and from the unlimed, but the difference was small. The average specific gravity of the oranges from the mulched and limed trees was 1.0012, and of those from the mulched trees, but not limed, was 1.0307. The specific gravity was calculated from the grading results, and not by individual determinations of separate fruits. The error in sizing by the grading machine would probably be compensated by the number of fruits included, as the entire crop was used in the calculation.

On the whole, the evidence obtained from these experiments does not show that the humus content of the soil correlates with orange production. Neither does the information obtained justify the statement that the humifying activity in the soil correlates with fruit production; but the results obtained would indicate that this point might be worthy of further study.

It appears from the results here presented that the most important function of organic matter as influencing orange production is not that of merely furnishing humus; that humus in itself is not the most important product of organic degradation for orange production. It appears from other work (*8*) that a more important function of organic matter is to make the plant food in the soil minerals more readily available. It was found that organic substances, especially in a freshly decomposing condition, dissolved plant food elements in appreciable amounts, even when the organic solvents contained practically no electrolytes. This function and that of promoting the biological activities of the soil seem to be more important rôles of organic matter in the soil than merely to furnish humus.

SUMMARY

This report presents a study on (*a*) the changes in humus content in soils in basins mulched with different organic substances, (*b*) the effect of lime on humus content in soils in mulched basins, (*c*) the relation of humus content in the soil to orange production.

By "humus" is meant the brown- to black-colored organic extract obtained from soil leached with 1 per cent hydrochloric acid to the absence of calcium and the soil residue boiled for two minutes in a 7.5 per cent sodium-hydrate solution.

Humus determinations in mulched basins in citrus groves showed a fluctuation in the percentage of humus from time to time.

The average percentage of humus increased more in basins on clay loam soil than in basins on lighter soil tubes, with manure and alfalfa as mulching materials.

Usually the percentage of humus in basins increased more when manure was used as mulch than when alfalfa was used as mulch. This seemed,

however, to be due more to the humus added with the manure, than to the greater "humification" of the manure over the alfalfa.

When manure alone was used as mulch in basins the increase in humus was greater than when lime was added with the manure.

In most cases when lime was added to alfalfa in basins greater increase in the humus content occurred than when alfalfa alone was used.

Blood, acid phosphate, bone meal, tankage, or sulphur did not show any appreciable influence on the changes of humus content in mulched basins.

It was not evident that there was any appreciable accumulation of humus in the lower depths of soil due to the leaching of humus from the surface foot of soil.

There was no evident correlation between the amount of humus in the soil in mulched basins and the amount of fruit on the trees.

There was no evident effect of lime on orange production in these experiments.

Alfalfa and bean-straw mulch in basins on the heavier soil types produced from 30 to 100 per cent more oranges per tree than manure mulch. Manure mulch produced more oranges per tree than either barley hay, sweet clover, bur clover, or pine shavings. These differences were obtained in the summer following the application of the mulches in the preceding fall.

Alfalfa mulch and manure mulch in basins on the lighter types of soil produced no observable differences on fruit production of lemons in the course of one year. This statement is based only on observation and not on picking records.

In all experiments so far conducted by this Office in the Riverside area, the mulched-basin system on the heavier soil types has produced favorable growth response in a few months. It usually takes longer to produce appreciable response on the lighter soil types.

It would appear directly from the work here reported, and indirectly from work elsewhere reported that the degradation products from freshly decomposing organic substances are more effective in orange production than the amount of "humus" formed. And the value of a given mulch does not necessarily depend upon its being a legume or nonlegume.

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RELATION OF KINDS AND VARIETIES OF GRAIN TO HESSIAN-FLY INJURY[1]

[PRELIMINARY REPORT]

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It has long been known that certain varieties of wheat (*Triticum* spp.) are injured less than others by the Hessian fly (*Mayetiola destructor*). Packard[2] mentions the Underhill, Mediterranean, Lancaster, and Clawson varieties as being noted for resistance. He states that the Underhill variety has been highly recommended for nearly a century.

Woodworth[3] made observations on 125 varieties of wheat grown at the California Experiment Station in 1886, 1887, and 1889, and noted the damage by Hessian fly in each. The Volo and Washington Glass varieties were not injured. Forelle; bearded wheat from Missoyen, Palestine; Polish; Bluegrass; Common March; Diamond; and Egyptian Imported were practically free from injury.

Roberts, Slingerland, and Stone,[4] in summarizing their observations on Hessian-fly injury in New York, conclude that

> the resisting power of varieties varies greatly

and that

> those with large, coarse, strong straw are less liable to injury than weak-strawed and slow-growing varieties.

Six varieties are mentioned which were not appreciably affected by the fly in 1901, although numerous other varieties in the same neighborhoods were much injured. These varieties were Dawson Golden Chaff, Prosperity, No. 8, Democrat, Red Russian, and White Chaff Mediterranean.

Gossard and Houser[5] made careful observations on 75 varieties of wheat and other grains grown at the Ohio Experiment Station in 1904, 1905, and 1906. They determined the percentage of stalks infested and of fallen straws. Their observations

> give but little support to the idea that there are immune varieties,

and they suggest that cases of supposed immunity may be explained by some other hypothesis. They state, however, that the most persistent

[1] Contribution from the Entomological Laboratory (Paper No. 30) and the Department of Agronomy (Paper No. 13) cooperating. This paper embodies some of the results obtained in the prosecution of projects No. 8 and 67 of the Kansas Agricultural Experiment Station.

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search has never located a single Hessian-fly egg on oats, although eggs were found on many grasses.

Since 1906 the relation between varieties and injury from Hessian fly has received scant attention from investigators. Presumably the work of Gossard and Houser has been accepted as disproof of the claims of earlier observers that some varieties are resistant and others immune.

Recently claims of immunity put forth by growers of certain varieties, general observations by farmers in eastern and central Kansas that hard wheats are more susceptible to injury than soft varieties, and results of experiments at the Kansas Agricultural Experiment Station indicate that the subject is at least worthy of further investigation.

Experiments have been outlined to determine (1) the relative infestation and injury of different kinds, varieties, and strains of various small grain, and (2) why certain kinds and varieties are resistant or immune; or, if not, why they escape injury in some cases where others are badly injured. This paper is concerned primarily with the first problem.

EXPERIMENTAL DATA

The data reported in this paper were collected from 87 kinds and varieties of wheat (*Triticum spp.*), oats (*Avena sativa*), barley (*Hordeum spp.*), rye (*Secale cereale*), emmer (*Triticum dicoccum*), einkorn (*Triticum monococcum*), and spelt (*Triticum spelta*), planted in the Agronomy Nursery of the Kansas Agricultural Experiment Station in the fall of 1916. The different varieties were planted each in a row 50 feet long and 10 inches apart. Two plantings were made on each of two dates, September 15 and October 1. The soil was in excellent condition, moisture was plentiful, germination was prompt, and growth was normal in every respect. Hessian flies were numerous, and, as far as known, there was ample opportunity for all varieties to become equally infested. In this paper all varieties are tabulated in the order in which they were planted.

Eight of the varieties tested were from Australia (rows 1 to 8, inclusive, Table I), and had not been grown previously at Manhattan. The spring varieties and about half of the soft winter varieties had been obtained from various Experiment Stations in the United States in 1914 and had been grown at Manhattan for two years only before being included in this experiment. All of the hard winter varieties and about half of the soft winter varieties had been grown at Manhattan for several years and were thoroughly acclimated.

The relative number of eggs deposited on each variety was determined by taking five consecutive plants from the west end of each row and counting the total number of eggs on the leaves. The first count was made at the time of maximum deposition, September 25. Subsequent determinations were made for the early sown plots on October 2 and 7, and for the late sown plot on October 14. The total number of plants of each variety examined was 20.

The relative number of flaxseeds in each variety was determined by examining 50 consecutive plants of each row in each plot sown on September 15, and 25 consecutive plants of each row in each plot sown on October 1, making a total of 150 plants of each variety. All plants were taken from the western end of the plots, or, in other words, adjacent to those plants which were examined for eggs. The pertinent data for each variety are given in Table I.

That the Hessian fly is apparently able to discriminate between kinds and varieties of grain is shown by these data. For example, the total number of eggs per 100 plants in the early sown plots ranges from 40 for Culberson winter oats to 5,600 for Turkey winter wheat No. 2407. The proportion of plants on which eggs were laid varied from 20 per cent for Culberson winter oats and Michigan winter barley to 100 per cent for Tennessee winter barley and most of the varieties of wheat.

The close agreement in determinations made at different times, and the striking differences in the number of eggs laid on adjoining varieties indicate that the difference can scarcely be attributed to experimental error. Thus, Turkey winter wheat No. 2407, which showed the highest total number of eggs, also had the highest infestation on September 25, the second highest on October 10, when the second determination was made, and was among the highest on October 7, when the third determination was made. On the other hand, Culberson Winter oats, Michigan Winter barley, and einkorn had the lowest total infestation and the lowest on each date.

Row 12 (Polish wheat) had a total of 2,040 eggs per 100 plants, as compared with 280 for row 13 (einkorn). Row 15 (spring emmer) had a total of 660 eggs per 100 plants, as compared with 1,740 for row 16 (Black Winter emmer).

On the whole, the Hessian fly appears to have shown a preference for common wheat, as compared with barley, oats, einkorn, spring emmer, spelt, and durum wheat. Black Winter emmer, Poulard wheat, and Polish wheat were as heavily infested with eggs as many of the common wheats. Rye showed a very heavy infestation, the total number of eggs per 100 plants being 2,500, which is well above the average for all grains included in the test.

Varieties of the hard winter wheat class were more generally infested than soft winter wheat varieties. Thus, 27 varieties of hard winter wheat averaged 2,737 eggs per 100 plants, as compared with an average of 1,835 for 38 varieties of soft winter wheat. However, there are wide variations in each class. For example, No. 2408 and Mealy, which are soft, or semihard, varieties, were infested with 4,720 and 4,320 eggs, respectively, per 100 plants, showing almost as high an infestation as the most profusely infested varieties of hard wheat, and more than double that of some varieties. On the other hand, certain varieties of hard wheat, such as Defiance No. 2129, Red Winter No. 839, Improved Turkey No. 2382, and Pesterboden No. 205, had a lower infestation than the average of the soft, or semihard, varieties.

TABLE I.—*Relation between the kind and the variety of grain and the infestation of the Hessian fly*

Row No.	Variety of grain.			Grain planted on September 15.									Grain planted on October 1.		
	Kind.	Name.	Agronomy Department No.	Number of eggs per 100 plants.				Plants with eggs Sept. 25.	Average number of flax-seeds per 100 plants.	Plants with flax-seeds	Average number of eggs per infested plants Sept. 25.	Average number of flax-seeds per infested plant.	Number of eggs per 100 plants.	Number of flax-seeds per 100 plants.	Plants with flax-seeds.
				Sept. 25.	Oct. 2.	Oct. 7.	Total.								
								Per ct.		*Per ct.*					*Per ct.*
1	Common white wheat	King's Early	1,401	1,800	480	40	2,320	100	183	35	18.0	3.5	900	10	12
2	.....do	Bunyip	1,402	1,860	360	0	2,220	100	123	39	18.6	3.7	100	72	24
3	White spring wheat	Bobs	1,403	1,100	280	20	1,400	60	114	42	10.8	3.1	380	36	10
4	Durum wheat	Huguenot	1,404	320	60	0	380	100	34	15	3.2	2.2	100	8	6
5	.....do	Cleveland	1,405	160	160	200	520	60	118	34	2.6	3.0	40	10	6
6	Hard red wheat	Cedar	1,406	2,020	240	20	2,280	100	68	39	20.2	1.7	100	12	10
7	Durum wheat	Indian Runner	1,407	180	660	20	860	40	28	16	2.5	1.8	20	14	8
8	Common white wheat	Tuscany Solid Straw	1,408	620	1,260	100	1,980	100	157	36	6.2	4.4	580	.....	.....
9	Durum wheat	Kubanka	298	420	0	0	420	60	35	13	7.0	2.8	20	20	14
10	Hard red spring wheat	Marquis	297	740	180	80	1,000	100	71	32	7.4	2.5	440	.....	.....
11	Poulard wheat	.....	.....	1,980	40	20	2,040	100	35	11	19.8	3.8	280	6	4
12	Polish wheat	.....	.....	1,920	40	80	2,040	80	46	20	24.0	2.4	0	4	4
13	Einkorn	.....	.....	220	40	20	280	60	0	0	3.6	0	0	0	0
14	Spelt	.....	.....	460	120	0	580	80	55	26	5.7	1.9	380	2	2
15	Spring emmer	.....	.....	460	200	0	660	80	0	0	5.7	0	100	0	0
16	Winter emmer	Black Winter	.....	1,500	140	100	1,740	80	144	50	18.7	3.4	280	34	32
17[a]	Winter oats	Winter Turf	5,165	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
18	.....do	Culberson Winter	5,158	40	0	0	40	20	0	0	1.0	0	0	0	0
19	Winter barley	Tennessee Winter	8,042	220	0	0	220	100	1	1	2.2	1.0	0	0	0
20	.....do	Michigan Winter	7,096	120	40	0	160	20	13	5	6.0	1.5	0	0	0
21	Rye	Rye	.....	2,400	120	0	2,520	100	0	0	24.0	0	60	0	0
22	Hard red winter wheat	Kanred	2,401	1,960	160	20	2,140	100	140	42	19.6	4.3	160	36	22
23	.....do	Crimean Select	2,402	4,560	640	100	5,300	100	124	37	45.6	3.2	120	22	14

24	.....do.....	Turkey.....	2,407	4,820	680	100	5,600	100	258	57	48.2	3.2	460	68	34
25	Soft red winter wheat...	German.....	2,403	1,240	220	80	1,540	100	71	30	12.4	2.4	100	38	20
26	Hard red winter wheat..	Malakof	2,071	2,640	260	180	3,080	100	172	51	26.4	3.4	140	18	16
27	Soft red winter wheat...	Hybrid.....	2,408	4,020	560	140	4,720	100	55	25	40.2	2.6	160	32	20
28	Hard red winter wheat..	Kharkof.....	382	2,480	120	100	2,700	100	280	60	24.8	4.8	180	76	36
29	.....do.....	Ghirka.....	385	1,840	400	100	2,340	100	157	41	18.4	4.3	120	22	14
30	.....do.....	Turkey.....	570	4,380	460	40	4,880	100	191	51	43.8	3.8	100	36	26
31	.....do.....	Red Winter.....	839	1,340	200	160	1,700	100	158	50	13.4	3.5	220	46	26
32	Soft red winter wheat...	Mealy.....	69	3,860	380	80	4,320	100	165	47	38.6	5.0	20	14	12
33	Hard red winter wheat..	Malakof.....	113	3,460	200	40	3,700	100	219	48	34.6	6.6	140	42	52
34	.....do.....	Turkey Hybrid.....	196	1,320	280	140	1,740	100	289	65	13.2	5.4	140	50	29
35	.....do.....	Pesterboden.....	205	1,240	200	120	1,560	80	203	45	15.5	4.2	160	38	20
36	.....do.....	Beardless Hard Winter.	1,210	2,280	200	20	2,500	100	60	28	22.8	2.3	180	8	8
37	.....do.....	Kharkof.....	1,443	1,540	160	100	1,800	100	121	45	15.4	3.5	60	30	18
38	.....do.....	Turkey.....	2,039	2,820	440	20	3,280	100	151	46	28.2	3.5	20	70	34
39	.....do.....	.....do.....	2,042	2,120	120	20	2,260	100	173	57	21.2	3.8	60	54	30
40	.....do.....	.....	2,059	1,540	220	20	1,780	100	171	45	15.4	3.4	120	30	10
41	.....do.....	Bearded Fife.....	2,094	1,620	460	20	2,100	100	185	58	16.2	3.1	220	80	36
42	.....do.....	Red Winter.....	2,101	1,280	520	60	1,860	100	285	62	12.8	5.0	160	8	6
43	.....do.....	Alberta Red.....	2,105	1,980	200	100	2,280	100	228	55	19.8	4.7	200	56	32
44	.....do.....	Defiance.....	2,123	2,260	360	20	2,640	100	268	58	22.6	5.6	220	54	30
45	.....do.....	.....do.....	2,129	1,200	160	0	1,360	100	158	41	12.0	4.1	60	20	16
46	.....do.....	Red Winter.....	2,132	2,560	320	60	2,940	100	30	9	25.6	2.8	140	4	2
47	.....do.....	Champanka.....	2,136	2,480	200	80	2,760	100	141	42	24.8	3.5	120	34	28
48	Soft red winter wheat..	Red Hussar.....	64	480	440	220	1,140	100	93	47	4.8	1.8	200	52	16
49	Hard red winter wheat.	Hungarian.....	65	1,380	480	100	1,960	100	103	45	13.8	2.3	340	36	18
50	Soft red winter wheat..	Nigger.....	73	1,860	300	140	2,300	80	17	6	23.2	3.0	80	6	6
51	.....do.....	Fulcaster.....	83	1,380	420	100	1,900	100	19	10	13.8	2.5	220	6	2
52	.....do.....	Beechwood Hybrid..	100	1,060	300	360	1,720	100	3	3	10.6	1.0	0	2	2
53	.....do.....	Miracle.....	106	1,400	280	40	1,720	100	12	4	14.0	0	200	8	6
54	.....do.....	Marvelous.....	408	2,900	40	240	3,180	100	59	24	29.0	2.6	260	40	22
55	Hard red winter wheat..	Washington Hybrid..	194	540	480	80	1,100	80	73	27	6.7	2.5	200	0	0
56	.....do.....	Binkel.....	2,353	1,380	260	100	1,740	100	161	59	13.8	2.3	120	22	18
57	Soft white wheat.....	Michigan Bronze Selection.	2,365	1,380	60	120	1,560	100	15	10	13.8	2.0	140	0	0
58	Hard red winter wheat.	Improved Turkey...	2,382	800	520	80	1,400	100	201	54	8.0	4.0	140	32	24

a Did not germinate.

TABLE I.—*Relation between the kind and the variety of grain and the infestation of the Hessian fly*—Continued

Row No.	Variety of grain.			Grain planted on September 15.									Grain planted on October 1.		
	Kind.	Name.	Agronomy Department No.	Number of eggs per 100 plants.				Plants with eggs Sept. 25.	Average number of flaxseeds per 100 plants.	Plants with flaxseeds	Average number of eggs per infested plants Sept. 25.	Average number of flaxseeds per infested plant.	Number of eggs per 100 plants.	Number of flaxseeds per 100 plants.	Plants with flaxseeds.
				Sept. 25.	Oct. 2.	Oct. 7.	Total.								
								Per ct.		*Per ct.*					*Per ct.*
59	Soft red winter wheat	Bucanera	2,404	980	80	40	1,100	100	246	54	9.8	5.0	160	46	32
60	.....do	North Allerton	2,405	1,660	140	40	1,840	100	150	47	16.6	2.5	160	46	28
61	.....do	Currell Select	2,406	1,580	480	120	2,180	100	3	2	15.8	2.0	100	8	4
62	.....do	Harvest Queen	9	860	280	60	1,200	100	42	13	8.6	10.5	280	8	6
63	.....do	.....do	19	1,740	140	20	1,900	100	44	11	17.4	2.7	60	4	4
64	.....do	Alabama Bluestem	33	1,440	160	80	1,680	100	184	52	14.4	3.7	140	36	20
65	Soft white wheat	Hybrid	59	780	140	80	1,000	100	24	10	7.8	2.1	60	4	2
66	.....do	.....do	66	1,700	20	80	1,800	100	46	17	17.0	1.7	80	6	4
67	Soft red wheat	Valley	70	500	220	40	760	80	8	7	6.2	1.1	100	0	0
68	Soft red winter wheat	Rudy	77	960	160	120	1,240	100	6	4	9.6	1.6	60	2	2
69	Soft white winter wheat	Dawson Golden Chaff	78	2,600	420	80	3,100	100	3	3	26.0	0	80	0	0
70	Soft red winter wheat	Dietz	84	1,320	320	60	1,700	100	15	7	13.2	1.7	120	0	0
71	.....do	Currell Prolific	90	1,360	140	40	1,540	100	38	14	13.6	2.0	140	2	2
72	.....do	Bearded Purple Straw.	109	1,080	160	60	1,300	100	23	10	10.8	2.6	1,120	0	0
73	.....do	Gipsy	111	1,820	20	80	1,920	100	157	32	18.2	4.1	80	14	6
74	.....do	Jersey Fultz	116	880	200	80	1,160	100	71	23	8.8	3.0	280	0	0
75	Soft white winter wheat	Hybrid	198	1,320	160	80	1,560	100	87	34	13.2	1.7	300	6	6
76	.....do	.....do	201	1,860	40	0	1,900	100	90	36	18.6	3.0	40	16	12
77	.....do	.....do	203	1,980	200	60	2,240	100	52	22	19.8	2.3	60	2	2
78	White club wheat	Washington Hybrid	204	1,480	160	60	1,700	100	53	19	14.8	2.6	160	0	0
79	Soft red winter wheat	Illini Chief [a]	223	1,820	200	80	2,100	80	0	0	22.7	0	260	0	0
80	.....do	Zimmerman	2,084	860	240	100	1,200	100	207	65	8.6	2.9	140	24	18

81	.....do.....	Sibley New Golden..	2,088	1,300	140	220	1,660	100	202	57	13.0	2.7	600	36	16
82	.....do.....	Fultz.....	2,156	1,680	120	160	1,960	100	132	44	16.8	2.7	20	4	4
83	.....do.....	Currell.....	2,157	840	180	20	1,040	100	65	23	8.4	3.1	40	4	4
84	.....do.....	German Emperor....	2,161	1,320	160	240	1,720	100	298	61	13.2	6.6	80	30	20
85	.....do.....	Deihl Mediterranean.	2,169	1,780	180	80	2,040	100	48	12	17.8	4.5	80	0	0
86	.....do.....	....do.....	2,175	1,520	220	140	1,880	100	40	16	15.2	3.1	20	14	10
87	.....do.....	Poole.....	2,180	580	20	180	780	100	133	47	5.8	3.2	40	58	28

[a] Illini Chief is also known as Finley and Early Carlyle.

Perhaps the most significant result is the high mortality of the eggs and larvæ, and as a result the relatively low infestation with flaxseed of all varieties and especially certain ones. The figures are open to criticism, so far as the absolute mortality is concerned, since more eggs may have been laid on the ends of the rows, where the egg counts were made, than on the adjoining plants, which were used for the flaxseed determinations. However, this criticism would not hold for the relative mortality for the different varieties.

As will be seen, no flaxseeds were found in einkorn, spring emmer, Culberson Winter oats, rye, and Illini Chief wheat. Very low infestations, 5 per cent or less, were recorded for Tennessee Winter barley and for Beechwood Hybrid, Currell Selection, and Dawson Golden Chaff wheats. The data for rye and for Illini Chief and Dawson Golden Chaff wheats are especially significant, in view of the fact that 2,000 or more eggs per 100 plants were laid on each.

Of the hard wheats, Red Winter No. 2132 is especially worthy of mention, since it had only 30 flaxseeds per 100 plants, as compared with over 200 for other varieties of the same class. Only 9 per cent of the plants of this variety were infested with flaxseeds, as compared with a range of from 28 to 62 per cent for other varieties of this class.

In general, the data show a low, or no infestation, with flaxseeds for rye, barley, oats, durum wheat, Poulard wheat, Polish wheat, spelt, emmer, and einkorn. The average number of flaxseeds per 100 plants for 38 varieties of soft winter wheat was 76.1, as compared with 173.7 for 27 varieties of hard winter wheat, an increase for the latter of more than 225 per cent.

These conclusions are based on the rows planted on September 15, but essentially similar results were secured from those rows planted on October 1. The infestation on these plots was much lighter than in the former case, and the data are less conclusive. It will be noticed, however, that those varieties which were not infested or which show a low infestation in the first plantings exhibit a similar characteristic in the latter.

FIELD TESTS.—In 1915 a bushel of Illini Chief wheat was secured by the Entomology Department of the Kansas State Agricultural College and planted in four localities in the State where the Hessian fly was abundant.

The Illini Chief was practically free from injury in all cases. At Manhattan it showed less than a 1 per cent infestation, while Turkey wheat in an adjoining plot was infested practically 100 per cent.

An examination of the plants in the fall and spring indicated that the fly showed no preference for either variety, the eggs being equally numerous on both plots. In the Illini Chief the maggots were able to work their way down to the crown of the plant, but at this point development appeared to be arrested and the larvæ died.

At Winfield, Kans., the Illini Chief was sown in the center of a 40-acre field of hard wheat. Determinations in the spring showed that 95 per cent of the plants of hard wheat were infested, as compared with about 10 per cent for the Illini Chief.

In 1916, a plot of Illini Chief wheat grown by the side of Turkey wheat showed an infestation of from 3 to 5 per cent, as compared with 95 to 100 per cent for the Turkey variety.

While these tests appear to show that Illini Chief is somewhat resistant to the Hessian fly, it should not be assumed that it is the best variety to grow. In Kansas it is one of the least hardy of all winter-wheat varieties and will survive none but the mildest winters.

CONCLUSIONS

The Hessian fly is able to discriminate between different kinds and varieties of grain. Eggs were laid on all the kinds and varieties of grain studied, but very sparingly on winter oats, winter barley, einkorn, spring emmer, spelt, and durum spring wheat.

On the average, fewer eggs were laid on soft winter wheat than on hard red winter wheat, but exceptions in both cases were found.

There appeared to be a large mortality of eggs or larvæ on all kinds and varieties studied. This appeared to be greatest for rye, einkorn, spring emmer, winter oats, and Illini Chief wheat. Very few flaxseeds were found on winter barley, and on Beechwood Hybrid, Currell Selection, and Dawson Golden Chaff wheats.

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